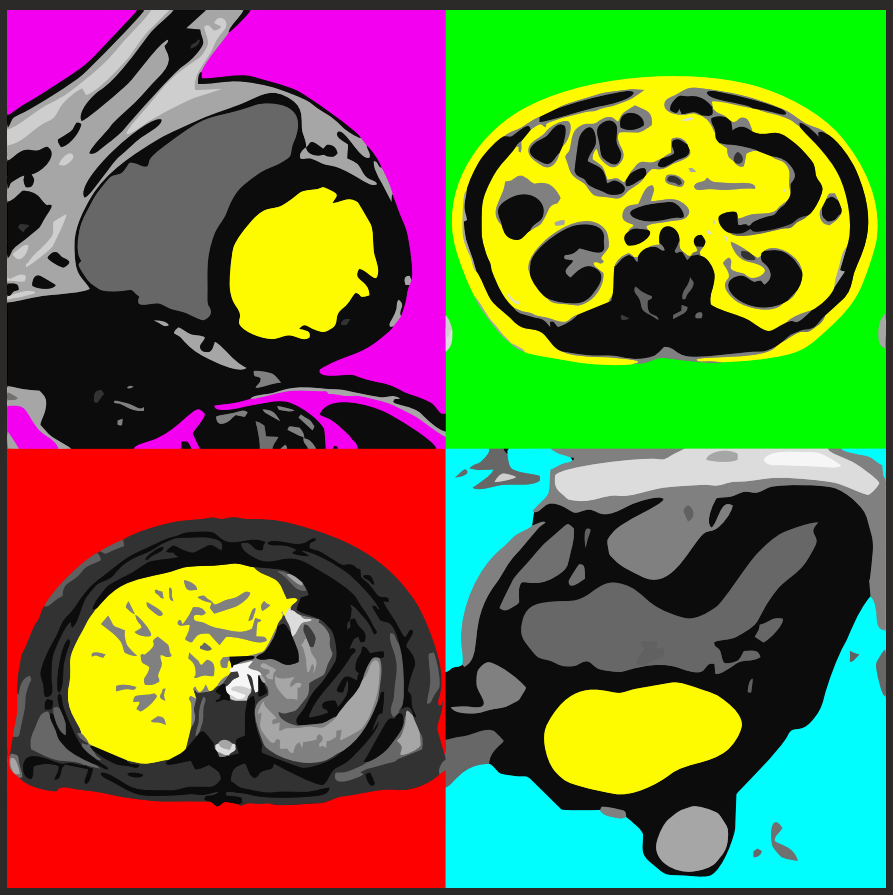
CARDIAC FUNCTION AND ECTOPIC ADIPOSITY IN METABOLIC SYNDROME

AN MRI AND ^{1}H-MRS STUDY

KRISTOFER NYMAN



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Kristofer Nyman

ACADEMIC DISSERTATION

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LIST OF ORIGINAL PUBLICATIONS

This thesis is based on the following original publications, referred to in the text by their Roman numerals.

I Marit Granér, Reijo Siren*, **Kristofer Nyman***, Jesper Lundbom, Antti Hakkarainen, Markku O. Pentikäinen, Kirsi Lauerma, Nina Lundbom, Martin Adiels, Markku S. Nieminen, Marja-Riitta Taskinen. Cardiac steatosis associates with visceral obesity in non-diabetic obese men. *Journal of Clinical Endocrinology & Metabolism* 2013, 98(3):1189-97. **Equal contribution*
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IV **Kristofer Nyman**, Marit Granér, Markku O. Pentikäinen, Jesper Lundbom, Antti Hakkarainen, Reijo Sirén, Markku S. Nieminen, Marja-Riitta Taskinen, Nina Lundbom, Kirsi Lauerma. Metabolic syndrome associates with left atrial dysfunction. *Nutrition, Metabolism & Cardiovascular Diseases* 2018, 28(7): 727-734.
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ABBREVIATIONS

3ch = three-chamber
AF = atrial fibrillation
ATP = adenosine triphosphate
BMI = body mass index
BSA = body surface area
CAD = coronary artery disease
CMR = cardiovascular magnetic resonance
Cr = creatine
CVD = cardiovascular disease
EDV = end diastolic volume
EF = ejection fraction
ESV = end systolic volume
FFA = free fatty acid
^{1}H-MRS = proton magnetic resonance spectroscopy
HF = heart failure
HDL = high-density lipoprotein cholesterol
HOMA-IR = homeostasis model assessment of insulin resistance
Hs-CRP = high-sensitivity C-reactive protein
LA = left atrium
LDL = low-density lipoprotein cholesterol
LV = left ventricle
LVGFI = left ventricular global function index
MetS = metabolic syndrome
MRI = magnetic resonance imaging
NT-proBNP = N-terminal pro-brain natriuretic peptide
NASH = non-alcoholic steatohepatitis
NAFLD = non-alcoholic fatty liver disease
^{31}P-MRS = phosphorus magnetic resonance spectroscopy
PCr = phosphocreatine
PFR = peak filling rate
SA = short axis
SAT = subcutaneous adipose tissue
SV = stroke volume
T2DM = type 2 diabetes mellitus
TG = triglyceride
VAT = visceral adipose tissue
VLDL = very low-density lipoprotein cholesterol

ABSTRACT

Overweight and obesity are major global health concerns affecting around 2 billion people worldwide. Abdominal obesity is particularly hazardous to health associating with metabolic syndrome (MetS), non-alcoholic fatty liver disease (NAFLD), and type 2 diabetes mellitus (T2DM). In MetS, ectopic fat accumulates around the viscera and into organs regularly containing only minor amount of adipose tissue. This cross-sectional study was conceived to discover effects of MetS and ectopic adiposity including myocardial steatosis on cardiac function in non-diabetic men free of known heart diseases. The gold standard non-invasive method for fat quantification, proton magnetic resonance spectroscopy, was used to measure hepatic and myocardial triglyceride (TG) content. Magnetic resonance imaging (MRI) was used to assess cardiac structure and function and to quantify epicardial, pericardial, subcutaneous, and visceral abdominal fat depots.

First, we examined the components of cardiac adiposity and their relationship to intra-abdominal ectopic fat deposits and cardiometabolic risk factors. MetS was associated with an increase in all examined ectopic fat depots. Myocardial TG was twofold increased in MetS. However, visceral adipose tissue (VAT) was the best independent predictor of cardiometabolic risk factors.

Secondly, we studied left ventricular (LV) diastolic function and its relationship with cardiac fat depots. MetS associated with LV diastolic dysfunction and concentric LV remodeling. However, myocardial TG content was not an independent predictor of LV diastolic dysfunction.

Thirdly, we studied the effect of different ectopic fat depots on LV structure and function in subjects with NAFLD and searched the role of hepatic fat content on cardiac steatosis. Epicardial and pericardial adipose tissue as well as myocardial TG content accumulated with increasing amount of both hepatic TG content and VAT. In addition, hepatic steatosis and VAT were associated with LV diastolic dysfunction.

Lastly, we studied left atrial (LA) structure and function in MetS and examined the association of different ectopic fat depots and cardiometabolic risk factors with possible LA dysfunction. MetS associated with subclinical LA dysfunction without enlargement of the LA. Moreover, LA dysfunction associated with several contributory factors of MetS including waist circumference, insulin resistance, dyslipidemia, and VAT, which was the best independent predictor of LA dysfunction. Notably, the role of myocardial TG content on LA function remained inconclusive.

In conclusion, MetS associates with significant changes in the structure and function of the heart. These changes are subclinical but may be followed by late complications such as heart failure or atrial fibrillation. The value of cardiovascular MRI may be early recognition of those obese subjects at risk for developing cardiovascular disease. Instead of body mass index, VAT is the best predictor of cardiac dysfunction. This study highlights the fundamental role of visceral obesity and hepatic steatosis directing their harmful effects to risk of cardiovascular diseases. The role of myocardial TG on cardiac dysfunction remains indeterminate. Therefore, cardiac energetics and the dynamic role of intramyocytic lipids, which are the primary fuel of the heart, merit further study.

TIIVISTELMÄ

Ylipaino ja lihavuus ovat maailmanlaajuisia terveysongelmia koskettaen noin kahta miljardia ihmistä. Keskivartalolihavuus on erityisen haitallista terveydelle liitännäissairauksiensa kuten metabolisen oireyhtymän (MBO), alkoholiin liittymättömän rasvamaksataudin ja tyypin 2 diabeteksen vuoksi. MBO:ään liittyy rasvan kertyminen sisäelinten ympärille (viskeraalirasva) sekä varsinaisen rasvakudoksen ulkopuolelle (ektooppinen rasva) kuten sisäelimiin.

Väitöskirjatyössä selvitettiin MBO:n vaikutuksia sydämen toimintaan ja sydämen rasvoittumisen roolia kardiometabolisena riskitekijänä miehillä, joilla ei ollut diabetesta tai tiedossa olevia sydäntauteja. Sydänlihaksen ja maksasolujen sisäistä rasva- eli triglyseridipitoisuutta mitattiin kajoamattomasti protonimagneettispektroskopian avulla. Sydämen rakennetta ja toimintaa, sydäntä ympäröivää epi- ja perikardiaalirasvaa sekä vatsan alueen ihonalais- ja viskeraalirasvaa tutkittiin magneettikuvauksella.

Tutkimme maksan rasvoittumisen, ihonalais- ja viskeraalirasvan sekä sydämen eri rasvaosioiden suhdetta toisiinsa. Osoitimme, että MBO:ssä ektooppisen rasvan määrä oli kasvanut ja sydänlihaksen rasvapitoisuus oli kaksinkertainen. Viskeraalirasvan määrä oli paras itsenäinen kardiometabolisten riskitekijöiden ennustaja.

Tutkimme sydämen vasemman kammion toimintaa ja sen yhteyttä sydämen rasvatiloihin. Sydämen vasemman kammion diastolinen toiminta oli alentunut MBO:ssä ja siihen liittyi konsentrista kammion uudelleenmuotoutumista. Vasemman kammion diastolisella vajaatoiminnalla ei ollut yhteyttä sydänlihaksen triglyseridipitoisuuteen.

Tutkimme sydämen ja muiden ektooppisten rasvakertymien roolia sydämen diastolisen vajaatoiminnan kehittymisessä ja maksan rasvoittumisen yhteyttä sydänlihaksen triglyseridipitoisuuteen. Epi- ja perikardiaalirasvan määrä sekä sydänlihaksen triglyseridipitoisuus korreloivat maksan ja viskeraalialueen rasvaan. Maksan rasvoittumisaste ja viskeraalirasvan määrä korreloivat lisäksi sydämen vasemman kammion diastoliseen vajaatoimintaan.

Selvitimme sydämen vasemman eteisen rakennetta ja toimintaa henkilöillä, joilla oli MBO, ja ektooppisten rasvakertymien sekä kardiovaskulaaristen riskitekijöiden vaikutusta niihin. Sydämen vasemman eteisen toiminta oli heikentynyt ilman eteisen koon muutosta. Vasemman eteisen vajaatoiminta oli yhteydessä useaan MBO:n osatekijään, kuten vyötärönympärys, insuliiniresistenssi, rasva-aineenvaihdunnan häiriöt ja viskeraalirasvan määrä, joista viimeksi mainittu oli paras itsenäinen ennustetekijä. Eteisen vajaatoiminta ei korreloinut sydänlihaksen rasvan määrään.

Löysimme sydämen magneettikuvauksella MBO-potilailta merkittäviä sydämen rakenteen ja toiminnan piileviä muutoksia, joiden mahdollisia myöhäiskomplikaatiota ovat sydämen vajaatoiminta ja eteisvärinä. Viskeraalirasvan määrä oli paras ennustetekijä piileville sydämen toiminnan häiriöille. Viskeraalirasvalla ja rasvamaksalla on tärkeä rooli lihavuuden haitallisten sydänvaikutusten systeemisenä välittäjänä. Osoitimme, että sydämen triglyseridipitoisuus lisääntyy MBO:ssä ja alkoholiin liittymättömässä rasvamaksataudissa, mutta sen rooli sydämen toiminnan häiriöiden kehittymisessä jäi avoimeksi. Sydämen energiatalous ja sydämen pääenergialähteenä toimivien lipidien dynaaminen rooli ovat otollisia kohteita jatkotutkimuksille.

INTRODUCTION

Origins of obesity can be traced back to prehistoric times when those of our ancestors who stored energy in the most effective way would best survive fast and famine [1]. While sedentary lifestyle and overabundance of calories now prevail in most parts of the world, the long-term result of natural selection has turned against us. Consequently, overweight and obesity have exploded to major global health concerns [2].

Obesity does not come alone but is often accompanied by a variety of comorbid conditions such as hypertension, dyslipidemia, type 2 diabetes mellitus (T2DM), non-alcoholic fatty liver disease (NAFLD), and metabolic syndrome (MetS). It was not until the 20th century when the connection between obesity and cardiovascular diseases (CVD) was solidified [3]. Now, we know that one of the main risk factors of coronary artery disease (CAD), heart failure (HF), and atrial fibrillation (AF) is obesity [4]. We don't, however, fully understand the pathophysiological mechanisms linking these diseases. Increasing evidence shows that the location of fat matters. Centrally obese people are particularly prone to develop MetS and cardiovascular complications of obesity and to have a deadly outcome [5]. In MetS, excess fat is stored not only around the visceral organs but also in non-adipose tissue such as liver, pancreas, skeletal muscle, and heart.

Liver is a key organ in lipid and carbohydrate metabolism and thus an essential player in obesity and MetS. The main organ to bear the deleterious consequences of these diseases is the heart. Modern non-invasive imaging techniques have revolutionized the quantification of ectopic fat accumulation in living humans [5]. With magnetic resonance spectroscopy (MRS), fat accumulation or lipid content of both liver and heart can be reliably quantified [6]. With magnetic resonance imaging (MRI), subcutaneous and the deep-lying visceral fat can be separated and the pericardial and epicardial fat embracing the heart can be measured accurately [6]. Additionally, MRI provides a three-dimensional assessment of the structure and function of the heart.

In the studies presented here, we have utilized MRS and MRI to study the role of MetS, ectopic fat depots, and clinical cardiometabolic risk factors in cardiac function in Finnish men free of known heart diseases. The themes of focus are distribution of ectopic fat, cardiac steatosis, left ventricular (LV) diastolic function, left atrial (LA) function, and influence of NAFLD.

REVIEW OF THE LITERATURE

1 Obesity

1.1 Prevalence and terminology

Overweight and obesity are major global health concerns of epidemic scale [2]. Obesity is generally estimated by the ratio of weight over height and the most commonly used anthropometric index is the body mass index (BMI) expressed in kilograms per meter squared. Among adults, a healthy weight corresponds to a BMI between 18.5 and 24.9 kg/m^2. BMI between 25.0 and 29.9 kg/m^2 is overweight, and BMI of ≥30.0 kg/m^2 is obese. The degree of obesity is further classified according to the BMI value where BMI of 30.0 to 34.9 kg/m^2 is class 1 or mild obesity, 35.0 to 39.9 kg/m^2 is class 2 or moderate obesity, and ≥40.0 kg/m^2 is class 3 or severe or morbid obesity [7].

In 2016, more than 1.9 billion adults (39% of men and 40% of women) were overweight and of these, 650 million (11% of men and 15% of women) were obese [2]. Between 1975 and 2016, the worldwide prevalence of obesity has nearly tripled. Today, most of the world's population live in countries where overweight and obesity kill more people than underweight [2].

In Finland, according to FINRISK 2012 health study, over 50% of adults were at least overweight and over 20% were obese [8]. The prevalence of overweight or higher was 65% in men and 46% in women. Regardless of gender, obesity was equally common.

1.2 Measurements of obesity

Even though BMI is a commonly used and suitable anthropometric index of obesity at the population level, it has been criticized because of its inability to differentiate between fat mass and lean body mass [9]. Thus, adults with normal weight but excess body fat may not be diagnosed as overweight or obese. Conversely, individuals with high levels of lean body mass may be misclassified as overweight or obese. Additionally, it is a relatively poor index of regional body fat distribution.

In 1980s it was reported that a simple index of regional body fat distribution, the ratio of waist-to-hip circumferences, was more strongly correlated to metabolic complications and to cardiovascular outcomes than the BMI [10, 11]. Afterwards, multiple studies confirmed that the regional distribution of body fat was more important than excess adiposity in initiating the CVD risk associated with a certain excess of body weight or fat [5].

During the last decades, waist circumference has been promoted as an ideal inexpensive clinical complementary measurement to BMI for assessing obesity. It is a simple yet effective way to assess for central obesity, with excellent correlation with abdominal imaging and high association with CVD risk and mortality [12, 13].

Consequently, definitions of the MetS have adapted waist circumference as a surrogate marker of abdominal or central obesity.

Waist circumference and BMI are not, however, very useful to comprehend the mechanisms by which body fat composition affect health risks. Waist circumference is primarily an index of total adiposity that is influenced by abdominal adiposity. An essential finding derived from studies involving cross-sectional imaging such as CT and MRI is that waist circumference, however, cannot distinguish visceral from subcutaneous abdominal adipose tissue [5]. Waist circumference may thus be increased due to abdominal subcutaneous or visceral adipose tissue (VAT) depots, or both.

The use of modern imaging enables precise and reliable quantification of differences in body fat distribution. The ability to store fat in various adipose tissue compartments can considerably differ from one individual to another. Generally, most of the body energy is stored in subcutaneous adipose tissue (SAT). Some individuals, however, can accumulate large amounts of adipose tissue in their abdominal cavity. An excess of intra-abdominal fat or VAT has been reported to be deleterious and associated with a set of metabolic abnormalities including insulin resistance, hyperinsulinemia, glucose intolerance, T2DM, atherogenic dyslipidemia (high serum TG, high LDL, and low HDL), inflammation, adverse cytokine profile, impaired fibrinolysis, increased risk of thrombosis, and endothelial dysfunction [5, 14]. Excess of VAT can be thus considered as a good marker of an altered cardiometabolic risk profile predictive of increased risk of T2DM and CVD.

1.2.1 Assessment of body fat composition

Various techniques have been used to assess body tissue composition and fat distribution. These techniques include, skinfold thickness measurements, underwater weighting, near-infrared interactance, air displacement plethysmography, and biometric impedance analysis.

Among imaging methods, dual-energy X-ray absorptiometry and ultrasound have been used in clinical setting to assess regional body fat distribution. CT and MRI have, however, revolutionized the study of fat distribution and body composition, and are therefore considered to be the gold standard for calibration of other methods estimating adipose tissue and lean body mass [7]. In both CT and MRI, the measurement of subcutaneous and visceral fat is typically performed at the level of lumbar spine.

1.3 Obesity complications

A wide spectrum of co-morbidities is associated with overweight and obesity [15]. T2DM, MetS, and CVDs, including hypertension, dyslipidemias, CAD, and HF are discussed in detail later in the thesis. Vascular risk factors all increase the risk for neurological complications such as stroke, dementia, and Alzheimer's disease. Obstructive sleep apnea and asthma are common complications of obesity regarding respiratory system. Gastrointestinal complications of obesity include non-alcoholic fatty liver disease (NAFLD), which is discussed later in the thesis,

unfavorable changes of the gut microbiome, and increased risk of pancreatitis, gallstones, and gastroesophageal reflux disease. Obesity is a major cause of colon, breast, endometrial, renal, and esophageal cancer. It is also associated with increased risk of gastric, pancreatic and gallbladder cancer, as well as leukemia. An obese person is in chronic low-level inflammatory state which has been proposed to be a central mechanism connecting obesity to its metabolic and vascular complications. Other common complications or risks of obesity include impaired renal function, urinary tract stones, gout, infertility, osteoarthritis, and depression.

As a result of a wide spectrum of increased morbidity, obesity associates with a decreased life expectancy [16].

2 Metabolic syndrome

2.1 Introduction

The importance of abdominal fat and association with hypertension and atherosclerosis was recognized as early as 1765 by the Italian anatomist Giovanni Baptista Morgagni [1, 17]. In the 1920s, the coincident relationships between metabolic abnormalities such as hyperglycemia, hypertension, and hyperuricemia were further documented [17]. These findings were important groundwork for the disease now known as metabolic syndrome, a clustering of coincident risk factors including abdominal obesity, impaired glucose tolerance, hypertriglyceridemia, low high-density lipoprotein (HDL) cholesterol, and hypertension [17]. The syndrome originally known as syndrome X was first conceptualized by Reaven in 1988 [18].

Two primary factors for the development of MetS are adult weight gain with body fat accumulation, and a tendency to store fat in intra-abdominal sites, including ectopic fat in liver, pancreas and heart [19]. MetS is strongly associated with a lifestyle characterized by physical inactivity and unlimited supply of food with high calorie and low nutrition content [19]. However, due to the high individual variation and genetic and epigenetic factors for both the components and expression of the syndrome, not all individuals at risk develop MetS [19].

The original idea of MetS focused on the crucial role of insulin resistance which is clearly an important and associated feature of MetS [18]. Before the establishment of harmonized criteria for MetS in 2009, it was debated whether MetS should be considered a true syndrome or a mixture of risk factors [20-22]. The concept of MetS was claimed to have an unclear definition, limited value in clinical practice, and there were doubts regarding its value as a CVD risk marker [20, 23]. One of the defending arguments for MetS was that the diagnosis of MetS provides a focus on the cluster of CVD and diabetes risk factors that require attention and it emphasizes the multifactorial nature of the risk for these diseases [22]. Other benefits of the diagnosis of MetS include the emphasis it places on central obesity and the ability of MetS to identify high-risk individuals at a young age [24]. It also provides a useful metric for the scale and progress of global obesity, diabetes and CVD epidemic [24].

Today, it is well accepted that the combination of components of MetS represent a pathologic state that substantially augments risk for the development of T2DM and CVD [17]. Thus, recently the focus has been on MetS as an epidemiologic tool related to CVD risk [17]. Because each component of MetS is an independent risk factor for CVD, together they produce a large variety of vascular and cardiac diseases [17].

2.2 Prevalence

MetS is a growing epidemic in parallel with the obesity epidemic. It has been estimated that ~30% of overweight and ~60% of obese men and women meet the criteria for the diagnosis of MetS [17]. In adults, the prevalence of MetS in most European countries is 10-30% and in the United States up to 40% [19].

2.3 Clinical criteria for MetS

Diagnostic criteria of MetS have been variably defined by a number of organizations [19]. The most commonly used criteria in Europe is adopted by International Diabetes Federation (IDF). IDF Task Force on Epidemiology and Prevention; National Heart, Lung, and Blood Institute; American Heart Association; World Heart Federation; International Atherosclerosis Society; and International Association for the Study of Obesity published a guideline to harmonize the definition of the MetS in 2009 [25]. This definition recognizes that the risk associated with waist circumference will vary in different populations. For the Europid people the suggested cutoff for waist circumference is ≥94 cm for men and ≥80 cm for women. Higher thresholds (≥102 cm for men and ≥88 cm for women) are used as the waist circumference cut points to identify MetS in the United States. Lower waist cutoff points varying in different countries are recommended for Asian population. Notably, the American values are consistent with the American definition of abdominal obesity which equate to a BMI of approximately 30 kg/m^2 (also WHO criteria for obesity) in males. The European values are, however, closer to a BMI of 25 kg/m^2 in males (WHO criteria for overweight).

In addition to waist circumference, four other diagnostic criteria for the diagnosis of MetS are: elevated TGs (≥1.7 mmol/l), reduced HDL cholesterol (<1.0 mmol/l in males, <1.3 mmol/l in females), elevated blood pressure (systolic ≥130 mmHg and/or diastolic ≥85 mmHg), and elevated fasting glucose (≥5.6 mmol/l). Drug treatment for these conditions are alternate indicators. The original IDF criteria for MetS was defined by large waist circumference plus two other findings. According to the 2009 harmonized criteria, waist can be considered as one of the five criteria and 3 abnormal findings out of 5 will qualify a person for MetS [25].

2.4 Insulin resistance

In muscle and liver, one main role of insulin is the regulation of glucose uptake. In adipose tissue, insulin promotes adipogenesis by increasing fatty acid uptake and esterification and the synthesis of TG and inhibiting lipolysis [26]. In insulin

resistant individuals, there is greater demand for insulin secretion by the pancreatic β cells to facilitate peripheral glucose uptake leading to hyperinsulinemia. In adipose tissue, the dose-response to insulin-lipolysis is declined, leading to adipose tissue insulin resistance. Adipose tissue insulin resistance has been found to increase proportionally to visceral and hepatic fat and associate with the severity of NAFLD [26].

Excess free fatty acid (FFA) overflow is the central cause of insulin resistance and metabolic dysfunction [26]. FFAs derive from adipose tissue, hepatic de novo lipogenesis, or overflow from plasma TG. Obese adipose tissue expansion occurs through the enlargement of adipocyte size (hypertrophy) or the increase in adipocyte number (hyperplasia) [26]. As adipocyte hypertrophy is correlated with insulin resistance, it has been hypothesized that adipose tissue becomes dysfunctional when it cannot expand in response to excess caloric intake, eventually leading to the storage of excess lipids into visceral fat and ectopic fat [26]. On the other hand, if the function and insulin-sensitivity of the SAT is preserved and it is able to expand through hyperplasia, the subject will be protected against the development of MetS despite the positive energy balance [5].

Most people with MetS have both abdominal obesity and insulin resistance [25]. Insulin resistance is closely related to impaired glucose tolerance, T2DM and risk of CAD, augmenting morbidity and mortality risk [19]. Compared to general population, the risk for T2DM in MetS is 5-fold [25]. All MetS patients do not, however, develop insulin resistance due to high individual variety and complex contributing factors. Specifically, about a third of patients with MetS have normal insulin sensitivity [19].

3 Ectopic fat accumulation

3.1 Introduction

Excess caloric consumption and sedentary lifestyle combined with unfavorable genotype and several environmental factors result in lipid overflow due to failure of SAT to expand and store the excess of circulating FFAs. As a result, ectopic fat accumulates around the viscera and into sites mainly containing only minor amount of adipose tissue, such as the liver, pancreas, skeletal muscle, and heart [5, 7]. The resulting metabolic consequences are the features defining MetS as previously described.

Ectopic fat deposits have been classified into those with local and those with systemic effects [27]. According to this, perivascular, myocardial, epi/pericardial, and renal sinus fat have mainly local unfavorable effects, whereas VAT, or fat in the liver or skeletal muscles have systemic effects due to the important role of these organs in glucose, insulin, and lipid metabolism. For this reason, both the amount and location of ectopic adipose tissue are highly important with respect to the cardiovascular morbidity and mortality. Pathophysiology of VAT accumulation is reviewed in the following paragraph, whereas cardiac fat depots and hepatic fat are revised later in the thesis.

3.2 Visceral adipose tissue

In addition to the role of insulin resistance discussed in the previous chapter, several theories have been proposed to explain the deposition and harmfulness of VAT. From the embryological aspect, intra-abdominal fat has its origin as brown adipose tissue exhibiting greater mitochondrial density and rates of lipolysis and glycolysis than the subcutaneous white adipose tissue [19]. Intraabdominal fat appears thus primarily to be involved in high-turnover distribution of FFAs to other body organs. When intraabdominal fat enlarges into a fat storage, however, metabolic complications arise.

One theory to explain the link between visceral obesity and cardiometabolic risk burden is called the portal FFA hypothesis. According to this, expanded visceral fat depot exposes, through its lipolytic activity, the liver to high concentrations of FFAs [28]. This leads to impaired liver metabolism and contributes to the hyperglycemic, hyper-insulinemic, and hyper-triglyceridemic state. Controverting the portal FFA theory, it has, however, been shown that 80% of FFAs found in the portal circulation are from systemic adipose tissue [29].

Another theory explaining visceral obesity is the overactivation of hypothalamic-pituitary-adrenal axis leading to an increased control of carbohydrate and lipid metabolism by glucocorticoids [30]. It has been shown that visceral adipocytes have more glucocorticoid receptors than subcutaneous adipose cells. Therefore, overactivation of hypothalamic-pituitary-adrenal axis may induce fat deposition in the VAT while concurrently inducing insulin resistance in the liver and in the skeletal muscle.

Gonadal steroids play a major role in the regulation of adipose tissue distribution, function, and storage [31]. In men, low testosterone levels are associated with higher adiposity and visceral fat deposition [32]. In women, estrogens are considered responsible for the gluteal–femoral fat distribution [33]. After menopause, a transition toward an android visceral fat distribution occurs, presumably due to decreasing estrogen levels [31]. The same phenomenon has also been reported in studies regarding transsexual patients receiving appropriate steroid hormone replacement therapy [5]. Estrogen and gluteal-femoral fat favoring adipose tissue distribution seems to protect women from type 2 diabetes [33]. On the other hand, although visceral fat deposition is more common in men, it seems to have more detrimental effects in women. In a recent population-based study, abdominal SAT and VAT were associated with insulin resistance to a similar extent in men, whereas in women particularly VAT was associated with insulin resistance and insulin secretion [34].

Overactivation of the endocannabinoid system is another disturbance in hormonal milieu associated with excess visceral obesity [5]. In obese individuals, endocannabinoids, released from adipose tissue and involved in feeding behavior, energy metabolism as well as glucose and lipid metabolism are increased via insulin resistance and inflammation. Increased plasma endocannabinoid levels may stimulate the overexpression of cannabinoid receptors in a pathophysiological manner contributing to excessive visceral fat accumulation and decreases in adiponectin level [9, 35].

It is estimated that genetic factors contribute about 30% of the observed variance in BMI but about 70% of the variance in fat distribution that relates more to the MetS [36]. However, also epigenetic mechanisms such as poor intra-uterine growth and maternal smoking (during pregnancy or early infancy) exist which can determine the preference to store excess body fat in intra-abdominal and ectopic sites already in very early life [19]. Recognized lifestyle factors that increase the intra-abdominal fat are weight gain, a diet high in saturated fat, smoking, inactivity, and excess alcohol intake [19]. Overweight subjects with a high level of cardiorespiratory fitness have been characterized by low levels of VAT in comparison with BMI-matched overweight individuals with low cardiorespiratory fitness [37]. Although smokers tend to have lower BMI values than nonsmokers, they have more abdominal adipose tissue and more insulin resistance than nonsmokers [38]. Another important correlate of individual varieties in VAT is ethnicity. Whites have a greater susceptibility of VAT deposition than blacks [39]. Asian populations are more prone to VAT deposition at lower BMI values, and therefore at risk to develop T2DM at lower BMI values than whites [5, 40].

4 Cardiac function in obesity and metabolic syndrome

4.1 Cardiovascular risk

Obesity has been linked to development of CVDs including atherosclerosis, CAD, HF, and AF [4]. Obesity increases the risk of CVD secondarily through its impact on the development and severity of comorbidities such as hypertension, dyslipidemia, and insulin resistance or diabetes. It is estimated that a single unit increment in BMI leads to 4% increased risk of ischemic stroke, 6% increased risk of hemorrhagic stroke, 6% increased risk of HF, and 4% increased risk of AF [4]. A 10 kg increment in body weight results in 12% increased risk of CAD, 3 mmHg higher systolic blood pressure, and 2.3 mmHg higher diastolic blood pressure [4].

Obesity is an independent risk factor for the development of CAD. A strong, inverse linear relationship between BMI and earlier age of first myocardial infarct has been reported [41, 42]. In a meta-analysis of 190 672 subjects, adults who were obese had an earlier onset of incident CVD, a greater proportion of life lived with CVD morbidity, and shorter overall survival compared with adults with normal BMI [43]. The authors concluded that “the obesity paradox” (meaning a greater longevity after diagnosis of CVD for obese and overweight individuals), appears largely to be caused by earlier diagnosis of CVD.

In a meta-analysis of 87 studies and 951 083 patients, MetS more than doubled the risk of CVD, myocardial infarction, and stroke [44]. MetS was associated with a 2-fold increase in risk of CVD mortality, and a 1.5-fold increase in risk of all-cause mortality. In the absence of T2DM, MetS was still associated with a 1.8-fold increase in risk of CVD mortality and stroke, and 1.6-fold risk in myocardial infarction.

The association between MetS and HF is strong [45]. It is estimated that obesity doubles the risk of developing HF after adjustment for associated co-morbidities

[46]. Furthermore, the prevalence of insulin resistance is up to 60% in patients affected by HF [45].

4.1.1 The 'healthy obesity' paradox

Although being overweight and obese are strong risk factors for CVD, significant individual differences are observed in the cardiometabolic risk profile of subjects within the same BMI category [9]. In some obese patients, favorable metabolic features such as high levels of insulin sensitivity and HDL-cholesterol as well as low levels of fasting triglycerides (TG) and fasting glucose were observed, and a unique obesity phenotype known as 'metabolically healthy obesity' was introduced [47]. Furthermore, some studies reported that metabolically healthy obesity was not associated with an increased risk of CV mortality compared with normal weight individuals [48]. The definition of metabolically healthy in these studies has, however, been variable, and moreover, they have had a relatively short follow-up period of 10 years or less [9].

In two recent meta-analyses, it was shown that metabolically healthy obese phenotype should no longer be considered as a benign condition. One analysis, including 61 386 people with a follow-up period of more than 10 years, reported that metabolically healthy obesity was associated with an increased risk for all-cause mortality and CVD events [49]. Another meta-analysis involving 299 059 individuals, demonstrated a 100% increased risk for CVD events in the metabolically healthy obese group [50].

Although younger individuals may appear to have metabolically healthy obesity, they may with increasing age develop insulin resistance, diabetes, dyslipidemia, and arterial hypertension, defining MetS [9]. Thus, one must not disregard the long-term CV hazards of being characterized by an apparently benign obesity phenotype and the necessity to follow these patients over the long-term.

4.2 Hemodynamic changes

Traditionally, excess adipose tissue has been associated with increased blood volume and cardiac output [3, 51]. According to this concept, the presence of excess adipose as well as non-adipose tissue results in increased blood volume. The increase in blood volume prompts venous return to the right heart and increases LV preload. Through the Frank–Starling mechanism, the increase in preload increases stroke volume. As the change in heart rate with obesity is small or absent, this ultimately leads to increased cardiac output. Simultaneously, the increased preload leads to increased ventricular wall tension which in chronic condition may lead to chamber dilatation and contribute to HF. Additionally, afterload is increased due to coexisting hypertension, further escalating myocardial demand, contributing to cardiac remodeling, left ventricular hypertrophy (LVH), and the development of HF.

Recently, this concept has been questioned and the relationship between obesity and LV structure and function seems to be more complex than presumed [52]. It has been reported that blood flow per unit of adipose tissue is relatively low

at 2 to 3 ml/min and is insufficient to fully account for the high cardiac output state. High cardiac output may not even be present in all forms of obesity. One explanation may relate to the role of body fat distribution. Recent findings suggest that in central obesity, cardiac output is lower and systemic vascular resistance is higher than in peripherally located obesity [53].

4.3 Morphological changes of the heart

In autopsy studies before the age of non-invasive cardiac imaging, elevated heart weight and left ventricular hypertrophy were main findings associated with obesity [3]. Echocardiography and CMR have revolutionized the non-invasive assessment of cardiac morphology in overweight and obesity, but data concerning the incidence of cardiac chamber enlargement and LVH has been inconsistent. Based primarily on echocardiographic evaluation, the following incidence values have been reported: increased LV mass (6%–87%), increased LV wall thickness (6%–56%), increased LV diastolic dimension (8%–40%), LA dilatation (10%–50%) and right ventricle enlargement (32%) [3]. The large variation of results attributes to variation in severity of obesity, duration of obesity, and presence or absence of associated morbidities. Echocardiographic data regarding cardiac remodeling in obesity has mainly pointed towards eccentric cardiac remodeling where both LV mass and EDV are increased [3]. However, several opposite findings reporting concentric remodeling have also been published recently [52, 54]. Notably, the commonly used method for measuring chamber wall thickness in one dimension for the assessment of LVH and remodeling index with echocardiography is more prone to errors than measuring LV mass with CMR.

In a population-based CMR study of 997 subjects, LV mass and EDV correlated with BMI as well, but out of these two, only LV mass correlated with VAT [55]. In another large population-based CMR study of 5098 participants aged 45 to 84 and excluded for clinical heart diseases, LV mass and EDV were positively associated with BMI for both sexes [56]. Increasing levels of obesity were associated with concentric LV remodeling, expressed by increased LV mass-to-volume ratio which was due to a greater increase in LV mass relative to LV EDV. In men, as opposed to women, association of concentric remodeling and overweight or obesity remained significant after adjustment for cardiovascular risk factors including hypertension.

CMR studies examining the role of MetS or relationship of VAT and other fat depots with LV morphology are limited [55, 57, 58], or have not comprehensively examined the impact of body fat distribution on LV concentric remodeling. In a study of 110 subjects, concentric LV remodeling was associated with abdominal obesity, and only 20% of the subjects presenting with concentric remodeling had LVH [58].

4.4 Left ventricular function

4.4.1 Systolic function

LV systolic function is typically preserved in obesity [3]. Most studies comparing LV ejection fraction in obese and lean subjects have reported no significant differences between these groups. Even in morbid obesity, severe LV systolic dysfunction is rare in the absence of co-existing heart disease. In severe obesity, exercise induced rise in LV systolic function is, however, restrained in the presence of LV hypertrophy [59].

4.4.2 Diastolic function

Normal diastolic function allows the LV to fill at rest and during exercise without abnormal increase in diastolic pressure [60]. Diastolic function is influenced by LV volume and geometry, contractile coordination, heart rate, LV relaxation and stiffness, and the pericardium. Diastolic ventricular function consists of four phases: isovolumic relaxation, early rapid filling, diastasis and atrial filling [3]. In a healthy heart, the majority of LV filling takes place during the early diastolic phase and is the result of a suction effect caused by myocardial relaxation. Atrial contraction is responsible of the late filling phase where a minority of the filling occurs. Myocardial relaxation is an active and energy-consuming process that is related to uptake of calcium from contracted myocytes via sarco/endoplasmic reticulum Ca^{2+}-ATPase [60]. Stiffness (elastance) which is a passive process, depends on LV morphology (geometry, mass), viscoelastic properties of the LV (fibrosis), and the properties of the pericardium. Diastolic dysfunction is associated with considerable abnormalities of these two closely linked processes; LV active relaxation and stiffness [60].

LV diastolic function can be assessed with various invasive or noninvasive imaging modalities [60]. Left-sided heart catheterization and the measurement of LV diastolic pressure is the standard of reference for assessment of LV diastolic function. This procedure cannot, however, be justified routinely due to its invasiveness. Trans-thoracic echocardiography is the most commonly used method in clinical practice because of its availability and excellent temporal resolution. Echocardiographic assessment of diastolic function is typically based on the measurement of trans-mitral flow velocity which has various pitfalls [60] and is reported to be inaccurate in cases of normal LV EF [61]. CMR is considered the gold standard for the assessment of LV volumes and systolic function due to superior image quality, especially over echocardiography, high reproducibility, excellent spatial and sufficient temporal resolution, and with minimal operator variability, and without limitations of acoustic window or choice of imaging volume [60, 62]. Analysis of LV volume versus time curve has proved to be a reliable method to assess diastolic dysfunction with CMR [63, 64].

LV filling pressure is frequently elevated in classes 2 and 3 obese individuals, and increases substantially with exercise, often exceeding the threshold likely to produce pulmonary edema [3]. In echocardiographic studies, LV diastolic filling has

been shown to be impaired in obese and MetS subjects relative to lean subjects [3, 65, 66]. Additionally, increasing body weight (BMI or severity of obesity) correlates with greater impairment of LV diastolic filling [3, 67]. Correlation of LV mass to impaired diastolic function has been variable [3]. CMR studies concerning diastolic dysfunction in obesity or MetS allowing precise LV filling pattern analysis are limited [14,15].

4.4.3 Obesity cardiomyopathy

Obesity cardiomyopathy may be defined as HF that is due entirely or predominantly to obesity [3]. Obesity cardiomyopathy occurs mainly in class 3 or super-obese patients who have usually been severely obese for more than 10 years. Common co-morbidities of obesity cardiomyopathy include obstructive sleep apnea, AF, and typical symptoms include fatigue, shortness of breath, and lower extremity edema [52]. Endomyocardial biopsies revealed cardiac myocyte hypertrophy in obese subjects referred for HF investigations [68]. In addition, patients with obesity were less likely (23.3% vs 64.5% in lean subjects) to have a specific etiology diagnosed for their HF, suggesting an obesity specific cardiomyopathy.

However, thorough assessment of comparative contribution of obesity to the development of HF is difficult. Obesity is a major risk factor for hypertension, which contributes to the development of CAD, and associates with abnormal LV geometry and subsequent HF. Obesity is also linked to other cardiovascular risk factors such as diabetes mellitus, dyslipidemia, and inflammation, and relates to a low level of cardiorespiratory fitness which itself is a potent risk factor for HF [52]. CMR studies on specific findings in obesity cardiomyopathy have not been reported.

4.5 Left atrial function and atrial fibrillation

LA function is important for maintaining optimal cardiac performance in three distinct phases. 1) During LV systole LA acts as a reservoir of pulmonary venous return. 2) At the early LV diastole, it serves as a conduit and its function is adjusted by LV diastolic performance. 3) At the late phase of LV diastole, LA acts as a pump improving LV filling. In HF, with sustained increases in LV and LA pressure, LA dilatation ensues, and LA contractile reserve becomes diminished. In end-stage HF, this leads to a change of LA to a passive conduit dictated by ventricular distensibility [69]. In AF, the pump function of LA is lost due to dyssynchronous contraction of cardiomyocytes.

AF is the most common cardiac rhythm disorder and is associated with significant morbidity and mortality, especially that arising from stroke and HF [70, 71]. Obesity represents the second highest population-based risk for AF after hypertension and it has been estimated that almost 1 in 5 cases of AF can be attributable to overweight or obesity [72]. Multiple studies have identified LA size as an independent predictor of AF [73]. In obese patients without AF, LA enlargement has been related to hypertension and LV dysfunction [3]. However, CMR data regarding LA function in MetS is very limited.

Atrial remodeling is defined as a spectrum of pathophysiological changes in atrial structure and function that occur in response to stresses imposed by conditions such as hypertension, HF, T2DM, and obesity [74]. Atrial remodeling may be subdivided to structural remodeling, as measured by LA size, and mechanical remodeling, as measured by LA function. Decreased LA EF, which is a functional index of LA, seems to be a better measurement to correlate with LV end-diastolic pressure than maximum LA volume [75]. Chronically increased LV filling pressure, obtained during invasive heart catheterization, results in LA remodeling over time [76]. Milder, often subclinical forms of atrial dysfunction may thus show decreased LA EF without LA enlargement.

The pathophysiological mechanisms of atrial remodeling and development of AF in obesity are, however, complex and remain elusive. In addition to major risk factors (male sex, HF, CAD, diabetes, sleep apnea, smoking, and excessive alcohol use), they include unfavorable genotype, impaired diastolic function causing atrial stretch, focal adiposity, and inflammation resulting in scar tissue [71]. As a result, structural, functional and electrical changes occur comprising the substrate for AF.

5 Cardiac adiposity

5.1 Introduction

Excess fat deposition involving the heart of obese persons was first described by Senac in 1783 [3]. In 1806, Corvisart described adipose tissue surrounding the heart and suggested that in obese people the heart was "oppressed by enveloping fat that sometimes caused sudden death". Laennec first established the fatty heart entity in 1819, distinguishing between "fatty surcharge on the surface of the heart" and "fatty degeneration in which muscular substance was transformed into fat exhibiting pallor like dead leaves". In 1933, Smith and Willius published a study of post-mortem cardiac findings of 135 obese adults. They found that heart weight was increased in proportion to the degree of obesity and was far greater than expected in extremely obese persons. Moreover, almost all subjects had excess epicardial fat, whereas myocardial fat content was normal in most individuals. In later half of the 20th century, the idea of coronary obstruction as the central pathogenetic mechanism of CVD supplanted the role of cardiac adiposity [77]. A renewed interest in cardiac adiposity has risen in the new millennium, particularly as the result of the rapid development in the field of noninvasive imaging, making it possible to quantify ectopic fat masses and contents with increasing levels of accuracy. Current findings indicate that adiposity, inflammation, and arterial obstruction are simultaneously operative in modulating tissue ischemia and plaque vulnerability [77].

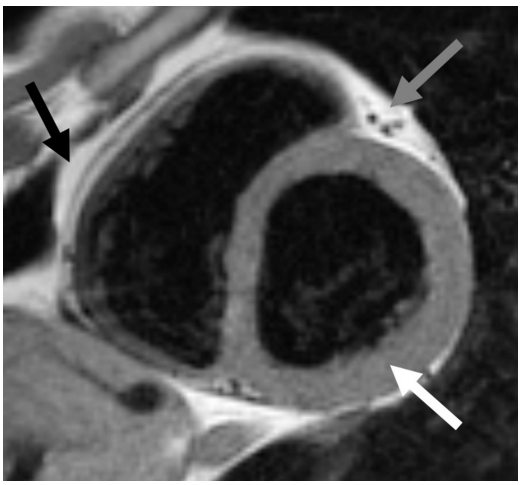

Figure 1. *A T1-weighted short-axis-oriented MR image demonstrates heart-related fat stores; fat in the myocardium (white arrow), epicardial fat (gray arrow), and pericardial fat (black arrow).*

5.2 Terminology

Heart-related fat can be divided into myocardial, epicardial, and pericardial fat (Fig. 1) [77, 78]. Myocardial fat content refers to TG droplets stored within the cardiomyocytes. Epicardial fat is the layer of adipose tissue between the myocardium and the visceral sheet of pericardium. Pericardial fat refers to the continuum of the thoracic or mediastinal adipose tissue sitting outside the parietal pericardium. In the literature, the term pericardial fat has different meanings. It has been used to describe epicardial and/or pericardial individually and it has been used to refer to both adipose deposits together. Sometimes, it has also been called paracardial fat [79]. To avoid confusion, the terms described above are used in the thesis similarly as in the original articles.

5.3 Myocardial fat

In proportion to its mass, heart is the most energy consuming organ in the human body [80]. Approximately 6 kg of ATP is utilized in the myocardium every day. Depending on the availability and demand, normal heart can generate its energy from lipids, glucose, amino acids, ketone bodies and lactate [80]. However, the dominant energy source of the myocardium is fatty acid oxidation. Circulating levels of FFA determine the cardiac fatty acid uptake which occurs through passive diffusion and active fatty acid transport proteins. In the resting state, a majority (70-90%) of fatty acids entering cardiomyocytes are rapidly used for ATP synthesis, whereas a minority (10-30%) of fatty acids are stored in the intracellular myocardial lipid pool [80].

Obesity and MetS are associated with ectopic deposition of lipids in non-adipose tissue including the heart. A formation of organs' steatosis is defined as the presence of an abnormally high levels of TG droplets in the cytosol of non-adipose cells (such as cardiomyocytes in the heart) [81]. Non-adipose cells are not, however, designed to retain large amount of TG for an extended time.

Consequently, the disproportionately high amount of TGs are eventually shunted into non-oxidative pathways resulting in the accumulation of toxic lipid species leading to condition called lipotoxicity [82]. Lipotoxicity has detrimental metabolic consequences including impairment of glucose metabolism, lipid oxidation and cell signaling, leading to oxidative stress, mitochondrial defects, apoptosis, and organ dysfunction [82]. Animal studies have provided evidence on a close relationship between cardiac lipotoxicity and impaired LV function [82].

In recent years, ^{1}H-MRS has allowed noninvasive quantification of cardiomyocytic TG content in living humans [83]. Based on ^{1}H-MRS evaluation, normal heart in lean individual contains less than 1.0% TG of the organ mass. Increased cardiac adiposity has been associated with obesity, impaired glucose tolerance, and T2DM where up to four-fold increases of myocardial TG content may occur [84-86]. In T2DM patients, myocardial TG content has been associated with LV diastolic dysfunction [87, 88] and concentric LV remodeling [85]. However, the mechanism behind this phenomenon has remained unresolved, and controversial reports have been published [86].

5.4 Epicardial and pericardial fat

5.4.1 Anatomy and embryology

Epicardial fat is a metabolically active fat deposit located within the pericardium and in direct contact with the myocardium and coronary vessels which provide its vascular supply [77]. Epicardial fat originates from a population of mesothelial cells that migrate onto the surface of the heart from the area of the septum transversum which is the embryological source of the diaphragm [89]. Epicardial fat, along with mesenteric and omental fat, develop from the splanchnopleuric mesoderm associated with the gut. In the normal adult, epicardial fat is concentrated in the atrioventricular and interventricular grooves and along the major coronary artery branches, and, secondarily, around the atria, over the free wall of the right ventricle and over the apex of the LV.

Pericardial fat lies on the external surface of the parietal pericardium within the mediastinum. It does not have a distinct outer border, because it is a continuum of the mediastinal adipose tissue. Pericardial fat originates from the primitive thoracic mesenchyme which divides to form the parietal (fibrous) pericardium and the outer thoracic wall [89]. Vascular supply of the pericardial fat differs from that of the epicardial fat. Pericardial fat receives its supply from multiple sources including the pericardiacophrenic artery, a branch of the internal mammary artery.

5.4.2 Function and clinical significance

Animal studies have demonstrated that epicardial fat shows higher lipogenic and lipolytic activities than other fat deposits [90]. Still, the physiology of lipid storage in human epicardial fat is poorly understood [91]. Epicardial fat has been suspected to serve as a local site of energy storage in the form of FFAs and as a protective buffer against high levels of FFAs and TG accumulation in the myocardium [79].

However, no direct evidence exist verifying the buffering action or demonstrating for reverse FFA movement from epicardial fat into the myocardium [91]. Furthermore, uneven distribution of epicardial fat over the surface of the heart would be unfavorable, if it was a source of FFA for the pumping heart.

Epicardial fat, located mainly around the coronary arteries in lean subjects, may serve a supportive, mechanical purpose, reducing vascular tension and torsion [77]. However, excess epicardial fat may cause adverse mechanical effects by tapering the myocardial relaxation and increasing myocardial stiffness.

In the Framingham Heart Study, epicardial fat was associated with obesity and higher LV mass [55]. Iozzo et al. have reported that epicardial fat associates with LV remodeling (although not independently) and visceral fat [85]. In echocardiographic studies, epicardial fat has been associated with MetS and LV diastolic dysfunction [78, 92]. Numerous studies have linked epicardial adipose tissue to increased AF burden [93].

In obese patients, epicardial adipose tissue secretes proinflammatory cytokines with a role in coronary atherogenesis [89]. Increasing evidence indicate that epicardial fat contributes also to clinical CAD. In a recent meta-analysis of 70 studies comprising 41 534 subjects, associations between CT-based assessment of epicardial adipose tissue volume and CAD were studied [94]. Epicardial fat was independently associated with coronary artery stenosis, myocardial ischemia, and major adverse cardiovascular events but not with coronary artery calcification.

Pericardial fat has been proposed to play roles in thermogenesis and supporting and mechanical purposes, such as attenuating vascular tension and torsion [95].

5.5 Assessing cardiac adiposity

5.5.1 Magnetic resonance spectroscopy of the myocardium

5.5.1.1 Introduction

The first pioneering acquisition of ^{1}H-MRS cardiac data was performed in 1984 in an intact, perfused rat heart by Ugurbil et al. [96]. The first human cardiac spectrum *in vivo* was recorded a decade later [97]. More disciplined studies utilizing localized cardiac spectroscopy *in vivo* became possible another decade later, after introduction of the two key technological elements: 1) sequences for spatial localization and 2) methods for the compensation and synchronization of the acquisition with respiratory motion and heart beating [98].

Currently, MRS allows the non-invasive biochemical analysis of *in vivo* changes in cardiac metabolism by detecting metabolites with proton (^{1}H) and phosphorus (^{31}P) nuclei in the human heart [99]. Cardiac ^{1}H-MRS is mostly used for the detection of myocardial TG. Other myocardial metabolites such as taurine, carnitine, and total creatine can also be detected and quantified by ^{1}H-MRS, but experimental procedures for these metabolites are technically more challenging because of low concentrations and overlapping frequencies [98].

Cardiac ^{31}P-MRS can be used to assess cardiac energy status by detecting the metabolites such as adenosine triphosphate (ATP) and phosphocreatine (PCr) [99]. However, relative MR sensitivity of phosphorus is about 15 times lower than that of a proton. For ^{1}H-MRS, usually the same radiofrequency (RF) coils can be used as for MRI, whereas ^{31}P-MRS requires dedicated ^{31}P coils and a second (multi-nuclei) RF channel [99].

5.5.1.2 MRS technique

As in conventional MRI, an RF pulse is applied, and the signal from the tissue is measured and Fourier transformed in MRS [100]. In MRI, frequency variation is used for spatial localization of the signal to a voxel to create a cross-sectional image. However, in MRS, the resonance frequency is used to separate and characterize the metabolites or chemicals within the voxel [100]. Further chemical information about tissue is captured from the signal intensity and the line width which may be used to detect the relative quantity of the chemical. The frequency shift or location of chemicals relative to that of water enables to generate qualitative and quantitative information about the chemicals that occur within tissues, forming the basis of MRS [100].

Nuclei suitable for nuclear magnetic resonance have an odd number of either protons or neutrons, which provides them with a quantum mechanical magnetic moment [101]. In a magnetic field, such nuclei align and precess at distinct quantized orientations and resonant frequencies. When excited with an RF field, flips between orientations occur and at the resonant frequency result in RF emissions and absorptions which can be detected with a tuned RF coil [101]. In a proton spectrum at 1.5 T, the metabolites are distributed between 63-64 MHz [100]. Resonance frequencies are commonly expressed as parts per million (ppm) to allow comparison of spectral measurements at different field strengths and to avoid the use of large numbers. In the ppm scale, the resonance of a chemical describes its position along the x-axis, where tetramethylsilane is commonly used as a zero reference (Fig. 2). Because the configuration of proton and electron interaction within chemical chains determines the resonance of a compound, it is possible to use the chemical shift to identify chemical compounds. The signal intensity, or height, of the metabolite peak, visualized as the amplitude measured along the y-axis, and the line width provide the area under a particular metabolite peak which can be used to quantify the amount of observed chemical compound within the voxel of interest [100]. There is no absolute scale (or unit) for the y-axis and the resulting metabolite concentrations are therefore usually reported as metabolite ratios to a presumably stable metabolite occurring naturally in tissue, such as water [100]. Following spectral acquisition, spectral analysis needs to be performed with dedicated software.

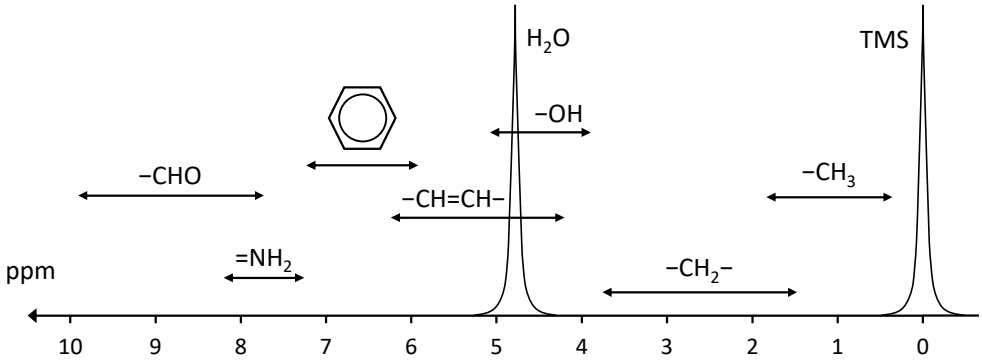

Figure 2. *^{1}H chemical shift ranges for some common organic moieties. TMS=tetramethylsilane. (Modified from Bottomley 2015, reproduced with permission of John Wiley and Sons.)*

Due to constant contractile and respiratory motion and blood flow, the heart is a particularly challenging target to image and to collect spectra. However, ECG-triggered and respiratory gated ^{1}H-MRS has proved to be a valid and reproducible method for non-invasive measurement of cardiomyocytic TG content in humans [83]. Cardiac ^{1}H-MRS is typically performed by single-voxel techniques such as stimulated echo acquisition mode (STEAM) or point-resolved spectroscopy (PRESS). The spectrum shows the resonances for water, TG (fat, lipids), and total creatine [102]. The spectrum is dominated by the signal from water which can interfere with the detection and quantification of signals from the metabolite moiety of interest. Such interference may occur when the dynamic range of the MR receiver is limited, when the concentration of moieties are <0.1% of water, and/or when they are complicated by broad spectral side-wings or intense motion artifacts spreading from the water signal peak [101]. The lipid (TG) spectrum consists of multiple peaks, where the dominant peaks are caused by the resonance of methyl ($-CH_3$) protons and methylene ($-CH_2$) in the TG molecule [100]. The size of the lipid peak relative to that of the water peak increases with the amount of TG content. To counter the dominance of the water peak and to weigh the relative metabolite content to the tissue water signal, spectra with and without water-suppression are acquired from the same voxel in the myocardium [99].

To prevent contamination from the epicardial fat, a voxel of interest is generally positioned in the interventricular septum [99]. The size of the voxel is usually large enough to average out local inhomogeneities in the myocardial fat distribution and to provide enough signal-to-noise ratio to permit a suitably short acquisition time for data collection [98]. If the voxel size is larger than the septal wall thickness, the spectrum may also include water signal from blood resulting in an underestimation of metabolite concentrations [99]. Another limitation of the technique is that septal TG content may not represent the global TG content because myocardial fat distribution may be heterogeneous in CVD [99].

5.5.1.3 Other applications of ^{1}H-MRS

Protons have the highest natural abundance among nuclei suitable for spectroscopy. Therefore, MRS applications in humans have tended to focus mostly on the ^{1}H nucleus [103]. The most widely studied organ is the brain where metabolites of numerous diseases including brain tumors, epilepsy, multiple sclerosis, and stroke have been investigated. In addition to the brain, prostate and breast have been other main organs for cancer studies [103]. The use of MRS in routine clinical practice, however, remains to be established. Regarding energy metabolism and diabetes, skeletal muscle has earlier been the main research target until the recent interest in cardiac MRS. Another important target for ^{1}H-MRS studies is the liver.

5.5.2 Imaging of epicardial fat

Most commonly used imaging techniques to measure epicardial fat are echocardiography, CT, and MRI [79]. Echocardiography is the cheapest and usually most easily available option for the evaluation of epicardial fat. It is limited by acoustic shadows especially in obese patients. Studies using echocardiography usually measure epicardial fat thickness on the free wall of right ventricle. Although echocardiography is highly operator-dependent, good intra- and interobserver reproducibility and moderately strong correlation with the thickness measurement and volume assessment of epicardial fat have been reported [79, 104]. However, epicardial fat is not evenly distributed over the heart and it is questionable whether thickness measurement on a single location can truly reflect the total epicardial fat burden.

Both CT and MRI provide accurate volumetric measurement of epicardial adipose tissue and have high intra- and interobserver reproducibility [79]. Epicardial fat assessment using CT and MRI has been shown to generate comparable results [105]. Advantages of CT imaging are that coronary artery calcium score may be calculated, and CT angiography may be performed to rule out and assess CAD non-invasively. As for cardiac MRI, a wide spectrum of applications is available from functional analyses to evaluation of different pathologies, including myocardial ischemia, myocarditis, and cardiomyopathies. Even though semi-automated techniques may be used for epicardial fat quantification, data analysis for both CT and MRI can be time consuming. Other disadvantages of MRI include high cost, limited availability and lengthy image acquisition. Radiation exposure is a major drawback of CT, as compared to echocardiography and MRI [79].

MRI provides an accurate tool to quantify epicardial fat. In T1- and T2-weighted images, epicardial fat shows as a high intensity fat layer between the intermediate signal myocardium and the low-intensity visceral pericardium. ECG-triggering or cine sequences are required to standardize the measurement for the phase of the cardiac cycle. Quantifying the whole epicardial adipose tissue volume is time consuming even with semi-automated techniques. Therefore, area-based measurements are commonly used [106]. Novel techniques such as 3D Dixon

sequence with fat-only images may prove useful for accurate computer-aided quantification of epicardial fat [107].

5.5.3 Imaging of pericardial fat

As mentioned before, due to various definitions and the lack of distinct surrounding fascia, quantification methods of pericardial fat are variable in the literature. For the analysis of pericardial fat, echocardiography provides very limited value. Contrary to the epicardial fat, where the fat exists in typical locations such as atrioventricular and interventricular grooves, pericardial fat is unevenly distributed over the parietal pericardium.

Both CT and MRI may be used for quantification of pericardial fat. The measured volume or area must be standardized, i.e. limited to a certain volume or a single slice [106, 108]. The same MRI techniques as described above for epicardial fat are valid also for assessment of pericardial fat.

6 Non-alcoholic fatty liver disease

6.1 Introduction

Patients with MetS often have excessive fat accumulation in the form of TGs in the liver and hepatic insulin resistance [109]. In the absence of other known causes of steatosis (e.g., alcohol, viruses, drugs), this condition is called non-alcoholic fatty liver disease (NAFLD). NAFLD is a spectrum of chronic liver diseases ranging from simple fatty liver to non-alcoholic steatohepatitis (NASH) which can progress to fibrosis, cirrhosis, and liver failure. Furthermore, NAFLD is an emerging risk factor of hepatocellular carcinoma which can develop even without cirrhosis in patients with NAFLD [109, 110]. Although NAFLD has been reported to be a common cause of incidentally discovered elevated liver enzymes, most NAFLD patients are asymptomatic and have normal transaminases [109].

6.2 Prevalence

NAFLD affects up to one third of the population worldwide, and its incidence is increasing rapidly because of the epidemics of obesity and T2DM [111]. Many different definitions of MetS, and the many methods of diagnosing NAFLD, make it challenging to estimate the coexistence of the two disorders [109]. The prevalence of NAFLD in subjects with MetS has been reported to increase 4-fold when compared with those without the disease and 35% of NAFLD subjects have been reported to have MetS [109, 112]. Each of the key components of MetS, insulin resistance, in particular, associates strongly with NAFLD [113].

6.3 Pathophysiology of NAFLD and relationship with MetS

Liver is a key organ in the control of carbohydrate and lipid metabolism [5]. In response to the energy demand of the body, liver releases substrates in the fasting period and stores glucose in hepatic glycogen and fatty acids in TGs postprandially under the regulation of insulin [114]. In the liver, two of the key components of MetS are produced; fasting plasma glucose and very low-density lipoprotein (VLDL) which contains most of the TGs present in serum [109]. In patients with NAFLD, the ability of insulin to normally suppress production of glucose and VLDL is impaired.

The excess accumulation of TG in the cytoplasm of hepatocytes is the hallmark of NAFLD [115]. This results from an imbalance between lipid acquisition and disposal [115]. In NAFLD, both processes are increased [116]. Sources of lipids contributing to fatty liver include FFA released into the circulation from peripheral adipose tissue, dietary fatty acids from intestinal chylomicrons, and lipids synthesized in the liver by de novo lipogenesis. Lipids are cleared from the liver mainly through either mitochondrial fatty acid oxidation or through secretion as a component of VLDL particles [115].

Overeating, especially of excess simple sugars, and physical inactivity predispose to both NAFLD and MetS [109]. De-novo lipogenesis, which is increased up-to three-fold in patients with NAFLD, converts simple sugars to fat in the liver [109]. In obesity, excess FFAs can enter the liver through the portal circulation [115]. Increased levels of hepatic FFAs induce increased lipid synthesis and gluconeogenesis. In patients with NAFLD or MetS, ability of insulin to inhibit glucose production is impaired, resulting in mild hyperglycemia, which stimulates insulin secretion resulting in hyperinsulinemia [109]. Furthermore, insulin resistance leads to failure of insulin to suppress both lipolysis and production of triglyceride-rich VLDL particles from the liver. Overproduction of large triglyceride-rich VLDL particles is a major contributory mechanism underlying the increase in serum TGs in patients with MetS and NAFLD [109]. This not only leads to lowering of HDL cholesterol but also to generation of small, highly atherogenic LDL particles.

Additionally, numerous other mechanisms (many of them also MetS-linked) contribute to the development of NAFLD. They include imbalance of adipokine production, overactivation of inflammatory chemokines and cytokines, various genetic factors, and disturbances in immune system and gut microbiome [116]. NAFLD is thus a complex disease arising from interactions between genetic and environmental factors and having a close relationship with MetS. NASH, which is the more severe form of the disease, is characterized by steatosis, hepatic inflammation, and hepatocellular ballooning and may include varying degrees of fibrosis [115].

6.4 Quantitative imaging of liver fat

6.4.1 Introduction

Traditionally, NAFLD or hepatic steatosis has been diagnosed based on histology obtained from liver biopsy with the definition of >5–10% of hepatocytes being fatty [6, 109]. However, modern MR techniques provide assessment of hepatic TG content non-invasively with lower sampling error (Fig. 3) [6].

6.4.2 Hepatic ^{1}H-magnetic resonance spectroscopy

The current gold standard to quantify hepatic TG content *in vivo* is ^{1}H-MRS [6]. It is the best technique to detect small amounts of fat. Commonly cited definition for NAFLD by ^{1}H-MRS is liver fat content ≥5.56%, given as weight fraction, which corresponds to about 10% of liver fat histologically [109, 117].

The same basic principles as earlier presented regarding cardiac ^{1}H-MRS can generally be applied to hepatic ^{1}H-MRS. Different from cardiac MRS, liver MRS may be performed with or without respiratory triggering [100]. A limitation of the technique is the variability of fat distribution throughout the liver [100]. Therefore, a single MRS measurement is not necessarily representative of the entire liver. However, T1-weighted in-phase and opposed-phase images may be used to recognize uneven liver fat distribution and aid in voxel positioning. Large vascular structures, bile ducts and focal lesions must be avoided when positioning the voxel of interest. Small voxel size may increase the risk for sampling error. However, the voxel size is typically at least two orders of magnitude larger compared to size of a liver biopsy sample.

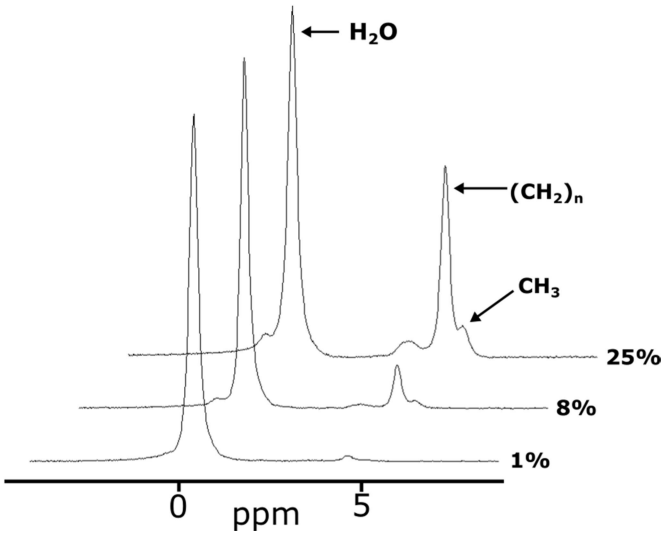

Figure 3. *Liver spectra of three subjects with varying degrees of hepatic steatosis.*

6.4.3 Other imaging techniques

Ultrasound is the most commonly used and most widely available noninvasive technique for detection of hepatic fat [118]. Steatotic liver demonstrates increased scatter and attenuation of sound waves compared with normal liver and produces characteristic pattern of hyperintense liver combined with blurry hepatic vessels and poor resolution of the diaphragm. The use of ultrasound is limited by poor penetration in obese individuals and lack of quantitative appliances, leading to great operator dependence and low sensitivity. However, in a recent study, subjective qualitative evaluation (4-point scale) of liver echogenicity was reported to have a relatively good correlation with ^{1}H-MRS measurement [119].

CT can be used for rapid assessment to quantify liver fat [118]. Degree of liver TG content may be estimated with attenuation values derived from CT used to estimate density. However, because scanning parameters such as voltage, tube current, and pitch and patient parameters such as BMI and the presence or absence of iron, iodinated contrast agents, or other substances vary from patient to patient, standardized quantitative measurement of steatosis is difficult [119]. Furthermore, CT requires exposure to ionizing radiation and for mild liver steatosis, sensitivity is limited [118, 119]. Dual-energy CT does not give added value as compared to mono-energetic CT in quantifying liver steatosis [119].

Recently, Dixon MRI techniques leveraging the difference between in-phase and out-of-phase water and fat signals have also been used in quantification of hepatic steatosis [6]. It enables the measurement of fat fraction relative to water content pixel-by-pixel, and the inhomogeneity of fatty infiltration may be visualized with color coded maps. The results are promising and comparable to ^{1}H-MRS as long as important confounding factors such as T1 bias, noise bias, T2* effects, spectral modeling of fat, and eddy currents have been addressed [119].

6.5 Association of NAFLD with CVD and cardiac function

NAFLD and MetS have significant overlap including the spectrum of diseases they predict [109]. MetS is an important predictor of NAFLD and NASH. NAFLD also predicts both T2DM and CVD. Although abdominal obesity is associated with NAFLD and MetS, whether visceral fat is causal or correlative of these disorders is controversial [5]. Emerging evidence suggests that NAFLD may be a CVD risk factor [111].

Many cross-sectional studies have shown that patients with NAFLD have an increased rate of CAD at coronary angiography as well as coronary artery calcium score detected by CT, independently of traditional cardiovascular risk factors [120]. In addition, relationship between the grade of NAFLD and the angiographic severity of CAD has been reported [120]. Several studies have reported that NAFLD, diagnosed with imaging techniques, is associated with an increased risk of fatal and non-fatal CVD events, independently of multiple cardiometabolic risk factors [120]. Furthermore, the severity of NAFLD is associated with higher risk of adverse CVD outcomes. NAFLD has also been associated with elevated risk of AF [120]. In a study of nearly 1 000 subjects with a long follow-up time, NAFLD was associated with an

approximately twofold increased risk of incident AF, independently of multiple confounding factors [121].

In echocardiographic studies, subclinical early LV diastolic dysfunction has been shown to associate with NAFLD in nondiabetic [122, 123] and in T2DM [124] subjects. MRS studies published on cardiac function in NAFLD have been limited and the results are controversial. Hepatic fat content and myocardial insulin resistance were associated in T2DM men in a study where LV function and morphology were not examined [125]. In men with uncomplicated T2DM, patients with increased intrahepatic fat content had reduced myocardial perfusion and lower myocardial phosphocreatine (PCr)/ATP ratio but similar LV function and morphology [126]. Analogously, nondiabetic men with increased intrahepatic fat content were reported to have impaired LV energy metabolism despite normal LV morphology and function [127]. Conversely, in a recent study, NAFLD did not affect PCr/ATP ratio or LV diastolic function [128].

AIMS OF THE STUDY

1. To explore components of cardiac adiposity and their relationship to intra-abdominal ectopic fat deposits and cardiometabolic risk factors in MetS (I).

2. To investigate whether LV diastolic function is impaired in MetS, and to examine how cardiac adiposity is related to the hypothesized LV diastolic dysfunction (II).

3. To assess the relationship of different ectopic fat depots on LV structure and function in subjects with NAFLD and to investigate the role of hepatic fat content on cardiac steatosis (III).

4. To investigate LA structure and function in MetS, and to examine the role of ectopic fat depots and cardiometabolic risk factors on LA dysfunction (IV).

METHODS

		Study	I	II	III	IV
Method	**Study object**	**Subjects (n)**	77	75	75	73
CMR	LV size and systolic function		X	X	X	X
	LV diastolic function			X	X	
	LA size and function					X
^{1}H-MRS	Myocardial TG content		X	X	X	X
	Hepatic TG content		X		X	X
MRI	Epi/pericardial fat		X	X	X	X
	Subcutaneous and visceral fat		X		X	X

Table 1. *Fat quantification methods and study objects.*

1 Study design and subjects

Publications I-IV are based on the data of Cardiac steatosis research project, a collaboration of HUS Medical Imaging Center (Radiology) and Heart and Lung Center (Cardiology), Helsinki University Hospital, and Diabetes and Obesity Research Program, University of Helsinki. The project was conceived in 2009 to primarily examine myocardial TG content as a new potential cardiometabolic risk marker. The study design is cross-sectional.

Study subjects aged 18 to 69 were recruited by advertisements in local newspapers. As the hormonal status and use of contraceptives modify lipid metabolism in women, only men were recruited. The study design is summarized and the rates of drop out at each stage are shown in study II, Fig. 1. The final study population comprised 77 (Study I), 75 (II-III) or 73 (IV) Finnish men of Caucasian ethnicity. Differences of subject count in studies I-IV were due to variation of available CMR data of interest.

T2DM related effects on CVD distinct from those of the underlying obesity and MetS. Therefore, T2DM (based on a 2-h oral glucose tolerance test or antidiabetic medical treatment) was part of the exclusion criteria. Stable hypertension treated with angiotensin-converting enzyme inhibitors, angiotensin II receptor blockers, low-dose diuretics, calcium channel blocker or selective beta blockers was allowed but subjects with untreated hypertension (blood pressure above 160/95 mmHg) were excluded due to possible confounding effect on cardiac function. Other exclusion criteria from the study included the following: known presence of or medical treatment for CAD, previous myocardial infarction, cardiomyopathy, sinus- or atrioventricular node dysfunction, asthma or chronic pulmonary obstructive disease; other known relevant chronic disease based on medical history, physical examination, and standard laboratory tests (blood counts, creatinine, aspartate aminotransferase, alanine aminotransferase, thyroid-stimulating hormone); and significant alcohol consumption (more than 20 grams per day). Due to common use in primary prevention, statins were allowed. Exclusion criteria included, however,

treatment with other lipid lowering therapy (e.g. fibrates). Smoking and up to 4-time upper limit of normal (70 U/L) elevated liver enzymes (alanine aminotransferase) were allowed. Five subjects were receiving medications for hypertension and five subjects received statins for dyslipidemia.

For the studies I, II and IV, subjects were divided into two groups based on cardiometabolic risk factors: those with MetS and those without MetS. To qualify for the MetS group, subjects must have waist circumference ≥ 94 cm in addition to two or more abnormal findings according to the harmonized definition of MetS [25]**.** The other subjects were classified as subjects without MetS. Subjects with waist circumference ≥94 cm without having additional MetS criteria and subjects with waist circumference <94 cm presenting with abnormal lipid profile were excluded. For the Study III, to explore the role of liver fat, we categorized the subjects into tertiles based on hepatic TG content: group 1 (n=24): low; group 2 (n=26): moderate; and group 3 (n=25): high hepatic TG.

1.1 Ethical statement

The Ethics Committee of the Department of Medicine, Hospital District of Helsinki and Uusimaa approved the study. Each subject provided written informed consent.

2 Demographic variables and biochemical investigations

Body mass index (BMI) was calculated by dividing weight in kilograms by the square of the height in meters (kg/m^2). Waist circumference was measured in the horizontal position at a level midway between the lower rib lateral margin and the iliac crest. Blood pressure was recorded as an average of five measurements using a BPM-200 monitor (Quick Medical, WA, USA) obtained in the sitting position after a 5 min rest. The subjects were classified as present, past, or non-smokers.

Blood samples were collected after an overnight fast. Total serum cholesterol, TGs, high-density lipoprotein cholesterol, very low-density lipoprotein cholesterol, FFAs, apolipoprotein A-I, apolipoprotein B, and high-sensitivity C-reactive protein were measured by Konelab analyzer 60i with Konelab TM kits (both from Thermo Fisher Scientific, Finland). The concentration of low-density lipoprotein cholesterol was derived from the Friedewald formula [129]. Measurement of aspartate aminotransferase, alanine aminotransferase, creatinine, N-terminal pro-brain natriuretic peptide, and thyroid-stimulating hormone were performed using standard laboratory techniques. Fasting and postload glucose were assessed by the hexokinase method (Gluco-quant, Roche Diagnostics, Basel, Switzerland) using either a Hitachi 917 or Modular analyzer (both from Hitachi Ltd, Tokyo, Japan). Serum insulin concentration was determined by double-antibody radioimmunoassay (Pharmacia RIA kit, Pharmacia, Uppsala, Sweden). The insulin-resistance homeostasis model assessment (HOMA-IR) index was calculated by using the formula: (fasting plasma glucose x fasting plasma insulin)/22.5 [130].

3 Cardiac imaging

3.1 Cardiac magnetic resonance imaging protocol

Cardiac MR images were acquired using a 1.5 T imager (Magnetom Avanto; Siemens, Erlangen, Germany) subject lying at rest in supine position. We used a multi-channel body coil for reception. Cine series were acquired in 4-chamber, 2-chamber, 3-chamber, and LV short axis orientations during breath hold using a retrospectively electrocardiographically gated steady state free precession gradient echo sequence [131]. A stack of short axis cine series (typically 12 slices) was obtained covering the LV from base to apex with typical imaging parameters of repetition time 50 ms, echo time 1.18 ms, flip angle 69 degrees, matrix 186 × 220, field of view 355 × 420 mm, slice thickness 8 mm, gap 2 mm, and temporal resolution 32–53 ms. Three-chamber oriented cine images were obtained with typical imaging parameters of repetition time 45 ms, echo time 1.05 ms, flip angle 64 degrees, matrix 156 x 192, field of view 300 × 380 mm, slice thickness 8 mm, and temporal resolution 44–50 ms.

3.2 Left ventricular analysis

Dedicated post-processing software (Argus; Siemens Medical Solutions, Erlangen, Germany) was used to perform a volumetric analysis of the LV. LV ejection fraction, mass, EDV, end-systolic volume (ESV), and stroke volume (SV) were measured, and both volume parameters and mass were reported as indexed to the subject's body surface area (BSA). An LV mass-to-volume ratio was calculated by dividing the LV mass by EDV (II). An LV global function index (LVGFI) (II) was calculated from the following formula: LVGFI = [LVSV/((LVEDV+LVESV)/2 + LV mass/1.05)]x100 [132].

Segmentation of endocardial borders was performed for all LV short-axis slices across all temporal phases to assess the time course of global volumetric filling. An LV early diastolic peak filling rate (PFR) was obtained from the LV volume versus time curve (II-III). LV EDV normalized values of PFR (PFR/LVEDV) were also calculated. The LV filling curve was visually inspected to identify the plateau between the early diastole caused by ventricular relaxation and the late diastole, the result of atrial contraction (II, Fig. 2A). The resulting diastolic plateau volume was divided by the EDV and the resulting percentile was reported as the ratio of early diastole. The physiological basis for measuring this index is that in the first phase of diastolic dysfunction, the proportion of LV filling in the early phase of diastole is reduced, and the contribution of left atrial contraction to LV filling is increased [62]. Diastolic dysfunction can be seen in LV volume kinetics as a depression of the diastolic plateau and as a shift from left to right in early diastole due to suppressed PFR (II, Fig. 2B).

3.3 Left atrial analysis

Three-chamber oriented images were used for measuring LA area in a single plane (IV, supplemental Fig. 2A). All phases of the cine images were inspected, and minimum and maximum LA area were planimetered using a diagnostic radiologic workstation (Impax 6 software, Agfa Healthcare, Mortsel, Belgium), and reported as LA 2D model. LA area measured in 3ch MR image has shown to be a reliable indicator of LA size [133] but volumetric CMR data based on 3ch images has not been reported. For 3D modeling of the LA, we used a prolate ellipsoid model which is commonly used for estimating LA volume in echocardiographic studies. Volume of the ellipsoid was calculated according to formula: $V = \frac{4}{3}\pi abc$, where πab = 3chA, area measured in the 3ch image, and c =0.5*h, where h equals maximum craniocaudal diameter of the left atrium in a short axis (SA)-oriented image at the midline of the LA (IV, supplemental Fig. 2B). Both measurements were done in end-systolic and end-diastolic images to obtain minimum and maximum LA volumes, and to calculate EF. Volume parameters are reported also as indexed to the subject's body surface area (BSA).

Comparison of LA assessment of 3ch prolate ellipsoid model and traditional Simpson's method, considered as gold standard, was performed for a group (n=10) of study subjects with sufficient data available for both methods. In these subjects, LA volume was planimetered from a stack of SA cine images with Simpson's method using dedicated software (QMass MR v.7.6, Medis Medical Imaging Systems, Leiden, Netherlands).

3.4 Adenosine stress perfusion magnetic resonance imaging

In participants who fulfilled the criteria for the MetS, we performed adenosine stress perfusion CMR to exclude myocardial ischemia and scar tissue. The rationale for this procedure was to exclude the potential interfering effects of CAD-associated myocardial scar tissue and/or altered cardiac function.

The adenosine stress perfusion MR was carried out in a 3.0 T MR imager (Verio; Siemens, Erlangen, Germany) according to a standardized protocol [131] including a first-pass perfusion module performed with a dynamic turbo flash perfusion sequence with typical imaging parameters of TR/TE/flip angle 199 ms/1.14 ms/12 degrees, matrix 192 x 180, slice thickness 8 mm, gap 20 mm, and a late gadolinium enhancement module acquired with a phase sensitive inversion recovery sequence with typical imaging parameters of TR/TE/flip angle 700 ms/1.16 ms/40 degrees, matrix 192 x 192, slice thickness 8 mm and gap 8.8 mm. Inversion time was set according to TI scout to null the normal myocardium. An intravenous adenosine infusion of 140 µg/kg/min was administered for five minutes under continuous ECG monitoring. Dynamic perfusion images were obtained in short axis (three positions) and 2-chamber orientations at four minutes after the onset of adenosine infusion during injection of 0.1 mmol/kg gadoteric acid (Dotarem, Guerbet, Villepinte, France) at a rate of 5 ml/s. Late gadolinium enhancement images were obtained 10 minutes after the administration of contrast agent in short axis, 2-chamber and 4-chamber orientations. Imaging data

was visually assessed to exclude any perfusion defects and myocardial late enhancement.

3.5 Quantification of myocardial triglyceride content

For measuring myocardial triglyceride (TG) content, we performed cardiac ^{1}H-MRS in a 1.5 T MR imager (Magnetom Avanto; Siemens) using a standard flex-coil for signal acquisition. A 36×24×10 mm^3 voxel was positioned in the interventricular septum (Fig. 4) using end-systolic cine images in three planes for localization. The localizer images and spectroscopic data acquisition were double-triggered to end-exhalation and end-systole, using Prospective Acquisition Correction navigator echoes (PACE, WIP-sequence, program version B17, Medical Solutions USA Inc, New York, New York) to control for respiratory movement and electrocardiograph-derived R wave to control for cardiac pulsation.

A PRESS sequence with echo time of 35 ms and repetition time >3000 ms was used for spatial localization and data collection. Navigator echoes were collected from the lung-diaphragm interface and the end-systole triggering was set at about 80% of the resting heart rate of the subject. The spectra were collected with and without water suppression, using 32 and 4 acquisitions, respectively, and analyzed with jMRUI v3.0 software (http://www.jmrui.eu) [134] using the AMARES algorithm [135] to determine water (4.7 ppm), methylene (1.3 ppm) and methyl (0.9 ppm) resonance areas (III, Fig. 1A). Myocardial TG content was expressed as a ratio of fat to water (%). We did not correct for methylene T2 relaxation due to lack of reliable data for cardiac application.

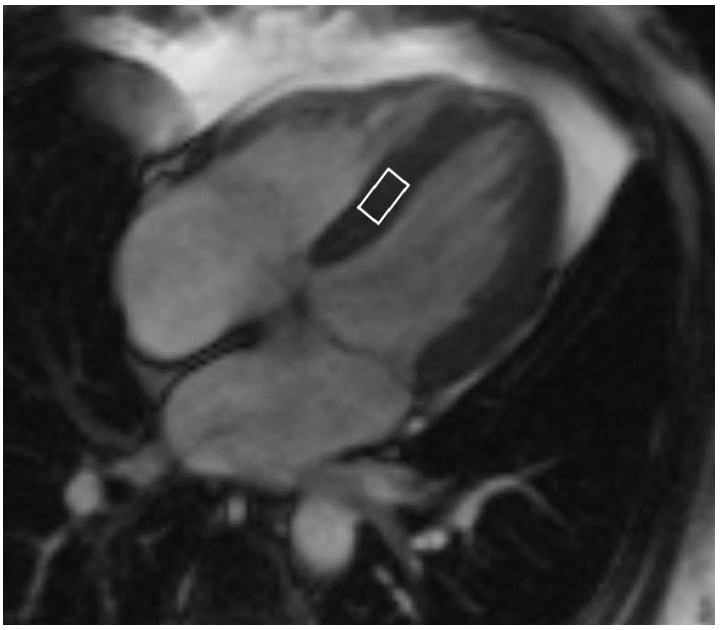

Figure 4. *Four-chamber oriented MR image of voxel positioning in the ventricular septum of the heart.*

3.6 Quantification of epicardial and pericardial fat

Segmenting the epicardial and pericardial fat planes is very time consuming and standardized measurement is challenging due to anatomical variety. In our preliminary data, epicardial and pericardial fat areas measured in a single 4-chamber view slice showed good correlation with epicardial and pericardial fat volumes measured with conventional Simpson method covering both right and left ventricles in a stack of short axis image slices (N=6, r=0.895, p <0.02). Therefore, we used epicardial and pericardial adipose tissue areas measured in a 4-chamber oriented image to estimate the amount of epicardial and pericardial fat, a method also used by others [106].

We inspected all phases of the cine images and performed the measurements in the single end-diastolic image using a standard radiologic workstation (Impax 5.5 software, Agfa Healthcare, Mortsel, Belgium). The areas of high intensity fat layers between the myocardium and the visceral pericardium (epicardial fat) and outside the parietal pericardium (pericardial fat) were measured (I, Fig. 1). Intra-thoracic adipose tissue outside the pericardium in the particular slice was included to the value of pericardial fat. A stack of short-axis oriented end-diastolic T1-weighted turbo spin echo images (Fig. 1, Review of the literature, Chapter 5.2) was also obtained (with typical imaging parameters of: repetition time 1050 ms, echo time 29 ms, flip angle 180 degrees, matrix 256 × 256, slice thickness 6 mm, and gap 1.5 mm), and they were additionally used to aid in epi- and pericardial fat separation as well as in water and fat separation, once needed. Intra- and inter-observer variability of epicardial and pericardial fat quantification was evaluated by two radiologists on separate occasions by the measurement of 20 (10 MetS and 10 without MetS) randomly selected study subjects.

4 Abdominal imaging

4.1 Quantification of hepatic triglyceride content

For measuring hepatic TG content, we performed ^{1}H-MRS in a 1.5 T MR imager (Magnetom Avanto; Siemens) using a standard body coil for signal reception. A 25×25×25 mm^3 voxel was placed in the middle of the right liver lobe avoiding large vessels and intrahepatic bile ducts (Fig. 5). Unsuppressed PRESS sequence with echo time of 30 ms, repetition time of 3000 ms and 4 averaged acquisitions was used to collect liver spectra during free breathing. We analyzed the spectra with jMRUI v3.0 software [134] and AMARES-algorithm [135]. Areas of water signal (4.7 ppm) and methylene (1.3 ppm) were determined using a line-fitting procedure (III, Fig. 1B). Signal intensities were corrected for T2 decay and hepatic TG content was calculated as methylene/(water + methylene) signal area x 100, and the values were further converted to mass fractions [136].

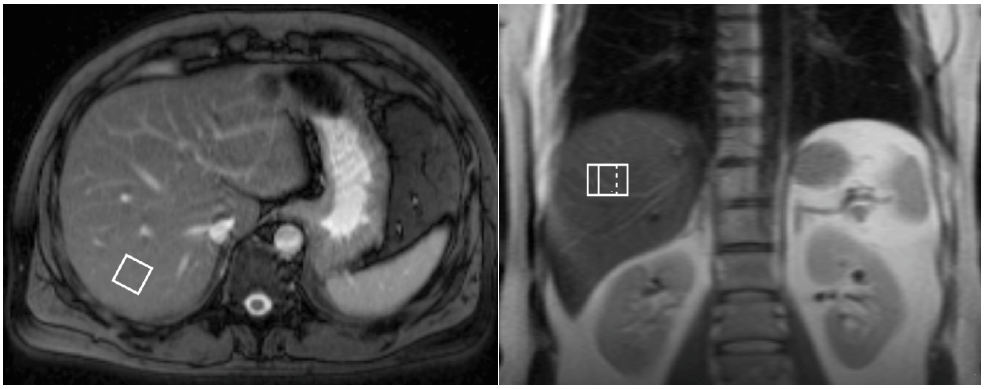

Figure 5. *Axial (left) and coronal (right) MR images of voxel positioning in the right lobe of the liver.*

4.2 Quantification of subcutaneous and visceral fat

Distributions of VAT and SAT were determined using a stack of 16 T1-weighted trans-axial 2-dimensional multislice spoiled gradient echo images acquired from a region extending from 8 cm above to 8 cm below the fourth and fifth lumbar intervertebral disks [137], using a standard body coil for image acquisition. MR images were analyzed using SliceOmatic v4.3 segmentation software. The areas of SAT (shown green in Fig. 6) and VAT (shown red in Fig. 6) were measured for each slice using a region-growing routine. The results were expressed as total volumes of SAT and VAT. We also calculated the VAT/SAT ratio as a metric of abdominal fat distribution [138].

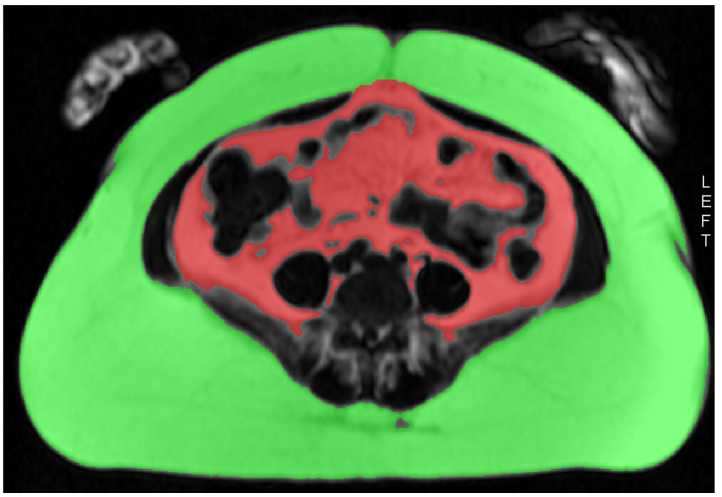

Figure 6. *Segmentation of the subcutaneous (green) and visceral (red) fat.*

5 Statistical analyses

We performed all statistical analyses with IBM SPSS Statistics (versions 19.0-24.0, IBM Corp., Armonk, NY, USA). Normality of continuous variables was analyzed by the Kolmogorov-Smirnov test. Logarithmic, square root or reciprocal transformation of variables was performed, if necessary. Data are presented as frequencies or percentages for categorical variables, as means ± SD for normally distributed continuous variables, and as medians (range) for skewed variables. Between-group differences (I, II, IV) were assessed by the Mann–Whitney U test, unpaired t-test, or the χ^2 test, as appropriate. For analysis of three groups (III), differences were examined by 1-way ANOVA, and P values for pairwise between groups differences were adjusted for multiple comparison in a post hoc Bonferroni t test. ANCOVA was applied to compare the means or medians of LV dimensions and function with adjustment for age. Levene's test was used to assess homogeneity of variances. If variances were not homogenous across the groups, nonparametric Kruskal–Wallis test was used.

For correlation analyses, Pearson or Spearman correlation coefficient was used depending on distribution of values. For analyses with distinct adjustments (i.e. age and smoking), partial correlation or univariable linear regression analyses were performed. Multivariable stepwise regression analyses were performed to determine independent predictors of cardiometabolic risk factors, LV diastolic function, and LA function (I-IV). Details of correlation analyses as well as univariable linear regression and multivariable regression analyses are described in each original publication.

Intra- and inter-observer variability of evaluation of epi- and pericardial fat was assessed via intra-class correlation coefficients (ICC) (II). Absolute agreement ICCs were calculated via a two-way mixed model for single measures. Three-chamber-based LA volume assessment and SA-based Simpson's method were compared using Pearson correlation and Bland-Altman analysis (IV).

For all analyses, $p<0.05$ was considered statistically significant.

RESULTS

Clinical and biochemical characteristics of the study groups (Publications I, II, IV)

Clinical and biochemical characteristics are summarized in Table 2. Subjects with MetS (n = 37) were, on average, 7 years older than subjects without MetS (n = 40) and included more current smokers. As expected, participants with MetS had greater waist circumference and BMI than controls. Subjects with MetS had higher fasting serum glucose and insulin, and HOMA-IR index respectively than control subjects. In the MetS group, 24 subjects had normal glucose tolerance, 4 had impaired fasting glucose, and 9 had impaired glucose tolerance. All individuals in the non-MetS group had normal glucose tolerance. Comparison of the serum lipid profile between the groups showed notable differences. In subjects with MetS, fasting plasma TGs were elevated on average 3-fold higher than in the control group. Additionally, higher total cholesterol and LDL, and lower HDL were observed in the MetS group compared to controls. Furthermore, systolic and diastolic blood pressure, and Hs-CRP were higher in subjects with MetS than in controls. NT-proBNP levels were comparable between the groups.

Table 2. *Summary of clinical and biochemical characteristics and fat compartments.*

	MetS present	**MetS absent**	***p***
N	37	40	
Age (years)	47 ± 6	40 ± 8	<0.001
Current smokers (N, %)	13 (35)	5 (12)	0.019
Body mass index (kg/m^2)	30.9 (24.2-42.5)	23.4 (17.6-29.8)	<0.001
Waist circumference (cm)	107 (94-135)	87 (71-94)	<0.001
Systolic blood pressure (mmHg)	132 ± 14	115 ± 10	<0.001
Diastolic blood pressure (mmHg)	88 ± 9	74 ± 6	<0.001
Total cholesterol (mmol/l)	5.3 ± 0.74	4.4 ± 0.79	<0.001
LDL (mmol/l)	3.3 ± 0.71	2.5 ± 0.66	<0.001
HDL (mmol/l)	1.0 ± 0.26	1.5 ± 0.39	<0.001
TGs (mmol/l)	2.2 (0.65-6.3)	0.78 (0.35-1.6)	<0.001
High-sensitivity CRP (mg/l)	1.8 (0.2-11.8)	0.2 (0.0-5.8)	<0.001
NT-proBNP (ng/l)	27 (5-151)	23 (5-74)	0.210
fP-ALT (U/l)	39 (20-260)	21 (7-59)	<0.001
fP-glucose (mmol/l)	5.8 (4.6-6.9)	5.0 (4.4-6.0)	<0.001
fS-insulin (mU/l)	9.3 (3.3-37)	2.9 (0.9-7.7)	<0.001
HOMA index	2.6 (0.8-8.0)	0.6 (0.2-2.0)	<0.001
Myocardial TG content (%)	0.90 (0.31-2.3)	0.44 (0.14-1.4)	<0.001
Epicardial fat (mm^2)	838 (385-1750)	520 (251-1130)	<0.001
Pericardial fat (mm^2)	1905 (615-6130)	562 (66-1580)	<0.001
Hepatic TG content (%)	6.59 (0.40-31.7)	0.73 (0.17-4.45)	<0.001
VAT (cm^3)	3300 (1260-5740)	843 (67-3170)	<0.001
SAT (cm^3)	4820 (2216-9354)	1770 (284-4020)	<0.001
VAT/SAT ratio	0.63 (0.26-1.64)	0.46 (0.13-1.23)	0.001

Data are expressed as means (± SD), medians (range) or as frequencies (%). MetS, metabolic syndrome; LDL, Low-density lipoprotein cholesterol; HDL, High-density lipoprotein cholesterol; TG, triglyceride; CRP, C-reactive protein; ALT, alanine aminotransferase; HOMA, the homeostasis model assessment insulin resistance; VAT, visceral adipose tissue; SAT, subcutaneous adipose tissue.

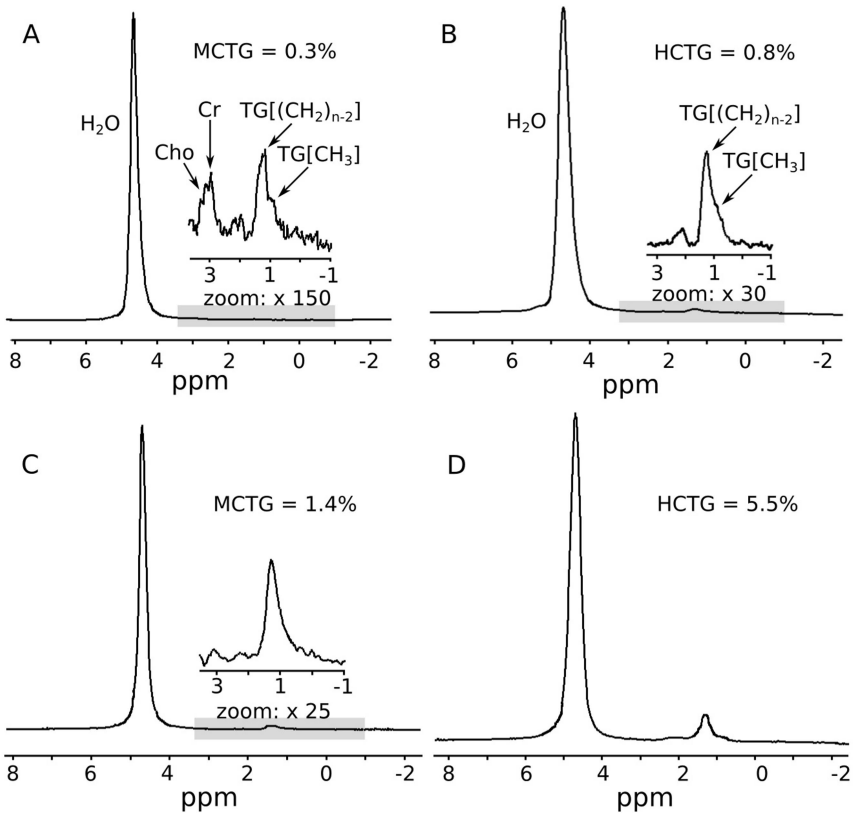

Figure 7. *Cardiac and hepatic spectra from 2 subjects: one with low (A) myocardial and (B) hepatocellular triglyceride (HCTG) contents, and the other with high (C) myocardial (MCTG) and (D) HCTG contents. Shaded areas have been zoomed above by factors 150, 30, and 25 for spectra A, B, and C, respectively. Reprinted with the publisher's permission from Publication III.*

MetS is highly associated with ectopic fat accumulation (I)

Measurements of ectopic fat deposits are shown in Table 2. Fig. 7 shows examples of cardiac and hepatic spectra. In participants with MetS, hepatic TG content was on average 9-fold higher than in controls (Fig. 8). Visceral and subcutaneous fat masses were higher in the group with MetS being increased by 3.9-fold and 2.7-fold compared to the group without MetS. Myocardial TG content was on average twice higher in MetS subjects. Similarly, epicardial and pericardial fat were markedly higher in subjects with MetS compared with subjects without MetS.

Visceral obesity is linked with hepatic and cardiac adiposity (I)

Univariate correlation analyses between ectopic fat depots and different clinical study parameters adjusted for age and smoking showed that VAT correlated with

hepatic TG content as well as with waist circumference and BMI (Fig. 9). Myocardial TG content, epicardial fat, and pericardial fat correlated with SAT and VAT, and hepatic TG content and the correlations seemed to be stronger with VAT than with SAT or hepatic TG content. Myocardial TG content, epicardial fat, pericardial fat, hepatic TG content, and VAT showed significant correlations with waist circumference, BMI, HDL, TGs, plasma insulin, and the HOMA index. Hs-CRP was linked to epicardial and VAT. Epicardial and pericardial fat were highly intercorrelated, however, neither correlated with myocardial TG content.

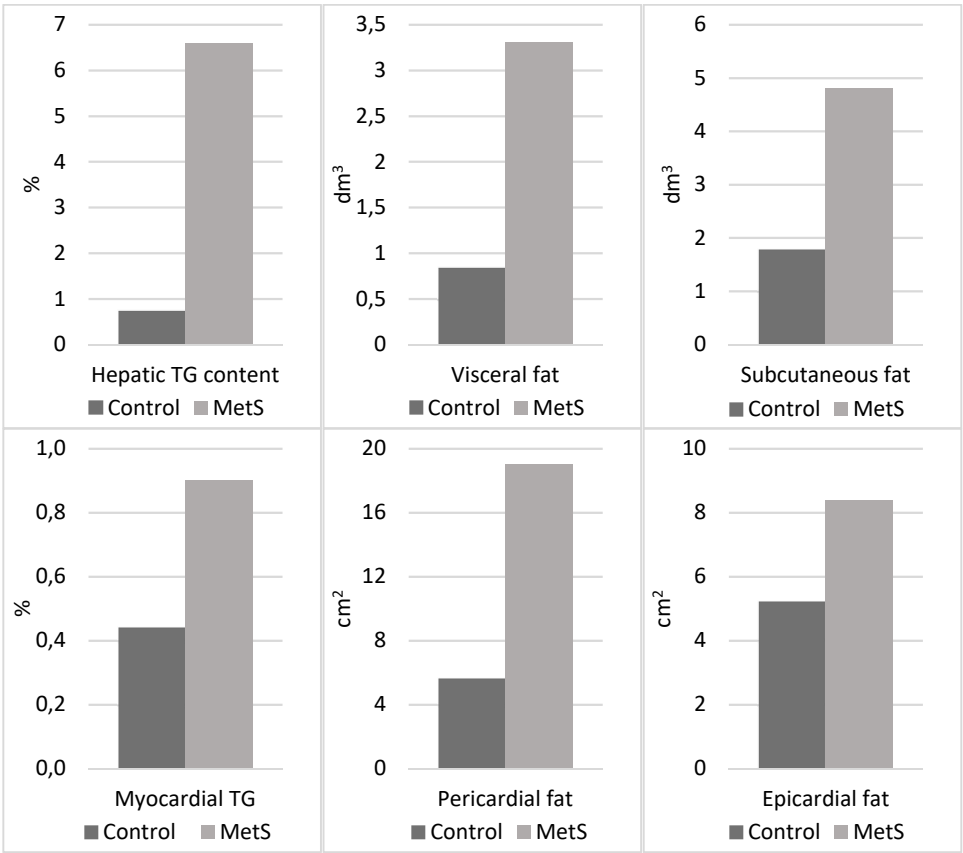

Figure 8. *Comparison of ectopic fat deposits between study groups. All correlations p<0.001.*

Visceral fat is the best independent predictor of cardiometabolic risk factors (I)

We performed stepwise multivariable regression analyses (Publication I, Table 3) to evaluate the impact of fat depots on individual cardiometabolic risk factors (serum TGs, HDL, fasting plasma glucose, plasma insulin, the HOMA index, and systolic and diastolic blood pressure). VAT was an independent predictor of TGs, HDL, plasma glucose, plasma insulin, and the HOMA index. Age and subcutaneous fat were independent determinants of systolic and diastolic blood pressure. However, cardiac adipose tissue depots did not correlate independently with cardiometabolic risk factors.

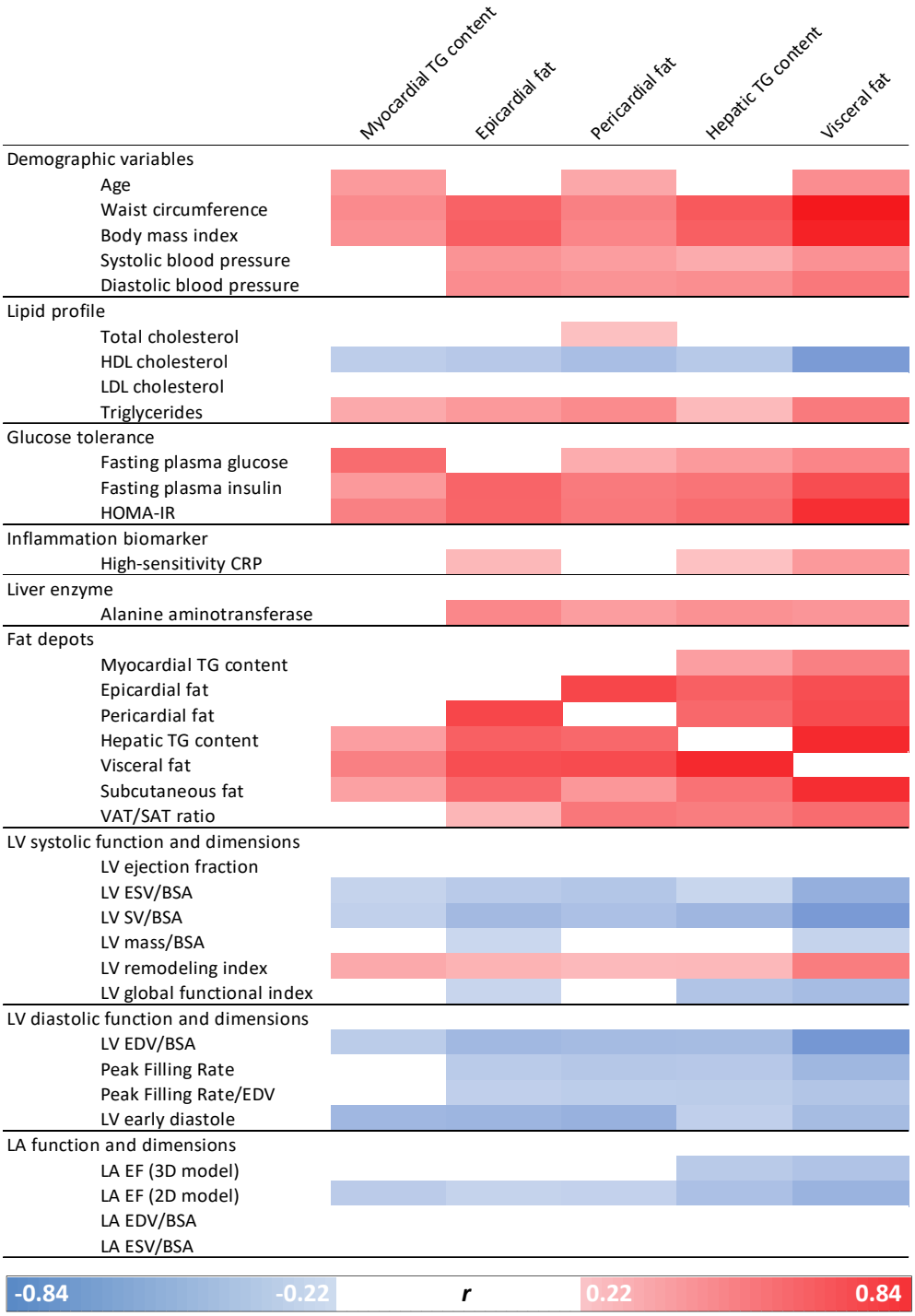

Figure 9. *Heatmap visualization of a summary of univariate correlations. Scale indicates strength of a relationship: red columns indicate positive, blue negative, and white non-significant correlations. Self-correlations are excluded.*

Reproducibility of fat quantification (II)

Intra-observer reproducibility for epicardial fat measurement in 4-chamber MR images was high with an intra-class correlation coefficient of 0.97 and 0.99 for pericardial fat, respectively. Inter-observer reproducibility showed an intra-class correlation coefficient of 0.91 for epicardial and 0.96 for pericardial fat.

With the aim of testing the repeatability of the cardiac ^{1}H-MRS with WIP-sequence, we repeated the sequence in five subjects with different degrees of myocardial TG content. Shim values and measurement parameters were kept unaltered. The measurements correlated (R^2 = 0.9975) with a coefficient of variation of 9.0%.

MetS associates with concentric LV remodeling and diastolic dysfunction (II)

Results of CMR measurements and analysis of LV function are outlined in Table 3. BSA- indexed values of LV ESV, EDV, and SV were smaller in the MetS subjects than in the subjects without MetS. LV volumes were within the normal range in both study groups, and differences remained significant after adjustment for the amount of exercise (data not shown). LV ejection fraction was normal in all participants and analogous between the study groups, indicating preserved systolic LV function. The BSA-indexed LV mass was also comparable between the groups. The LV mass-to-volume ratio was greater and the LVGFI lower in the MetS group compared to the control group, indicating concentric rather than eccentric LV remodeling. Diastolic dysfunction was associated with MetS as demonstrated by lower LV early diastolic PFR, PFR/EDV, and ratio of early diastole in subjects with MetS than in the controls (Fig. 10).

Table 3. *Summary of left ventricular dimensions and function.*

	MetS present	**MetS absent**	***p***
N	37	38	
LV ejection fraction (%)	61 ± 6	62 ± 4	0.745
LV ESV/BSA (ml/m^2)	25 ± 7	32 ± 5	<0.001
LV SV/BSA (ml/m^2)	40 ± 8	52 ± 7	<0.001
LV mass/BSA (g/m^2)	58 ± 9	62 ± 7	0.190
LV mass/EDV (g/ml)	0.88(0.66-1.32)	0.73 (0.61-0.90)	<0.001
LV global functional index (%)	41 (24-51)	44 (35-54)	<0.001
LV EDV/BSA (ml/m^2)	65 ± 13	84 ± 11	<0.001
LV peak filling rate (ml/s)	471 (238-909)	667 (329-1315)	0.002
LV peak filling rate/LV EDV (s^{-1})	3.37 (1.89-5.46)	3.75 (2.63-7.23)	0.023
LV early diastole (%)	68 ± 9	78 ± 8	0.001

Data are expressed as means (± SD), medians (range). MetS, metabolic syndrome; LV, left ventricular; ESV, end-systolic volume; BSA, body surface area; SV, stroke volume; EDV, end-diastolic volume.

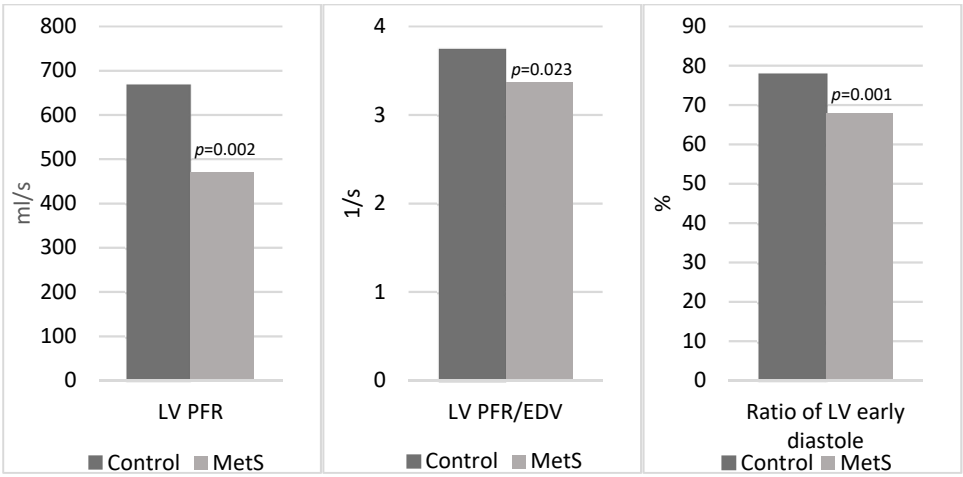

Figure 10. *Comparison of left ventricular diastolic function between study groups.*

Accumulation of epicardial and pericardial fat correlates with the degree of LV diastolic dysfunction (II)

Age-adjusted univariate correlation analysis (Fig. 9) revealed that the amount of epicardial and pericardial fat was inversely correlated with the parameters of diastolic function (PFR, PFR/EDV, and ratio of early diastole). Myocardial TG content correlated with the ratio of early diastole, but not with PFR. The LV remodeling index (mass-to-volume ratio) and BSA-indexed EDV, ESV, and SV correlated with the myocardial TG content, and with pericardial and epicardial fat.

Myocardial TG content is not independently associated with LV diastolic dysfunction (II)

We used a multivariate correlation analysis (II, Table 4) to further evaluate the interrelationship between the individual cardiac fat depots and LV diastolic dysfunction. Age and epicardial and pericardial fat were all independent determinants of PFR and PFR/EDV. Age and pericardial fat were also independent predictors of the ratio of early diastole. Interestingly, once the effect of age, waist circumference, body mass index, blood pressure parameters, and epicardial and pericardial fat were taken into account, myocardial TG content was not independently related to any parameter of diastolic dysfunction.

Clinical and biochemical characteristics of the study groups categorized based on hepatic steatosis (III)

Subjects were categorized into tertiles based on hepatic TG content: group 1: low (0.17-1.05%); group 2: moderate (1.17-5.16%); and group 3: high (5.45-31.74%) hepatic TG. Clinical and biochemical characteristics of the study groups are summarized in Table 4. Subjects with high liver fat were older, had higher BMI,

waist circumference, heart rate, and systolic and diastolic blood pressures compared with the other groups. There were more current smokers in individuals with high liver fat when compared with those with low liver fat. Participants with high liver fat had hypertriglyceridemia, lower HDL, higher LDL and a high concentration of apolipoprotein B, as well as elevated glucose and insulin levels and HOMA-IR, when compared with those with the low liver fat group. FFAs were comparable among the groups. Myocardial TG was 3-fold higher in the high liver fat group and 2-fold higher in the moderate liver fat group when compared with that in the low liver fat group. Furthermore, epicardial and pericardial fat depots were increased in the high liver fat group when compared with the other groups. As expected, VAT and SAT increased stepwise from the low to high liver fat group. The VAT/SAT ratio was comparable in the groups with moderate and high liver fat but significantly lower in the group with low liver fat.

Table 4. *Summary of characteristics and measurements categorized based on hepatic fat.*

	Low hepatic TG content	**Moderate hepatic TG content**	**High hepatic TG content**	***P***
N	24	26	25	
Age (years)	38 ± 7	45 ± 8*	47 ± 8*	0.002
BMI (kg/m^2)	23.2 (17.6-32.5)	25.8 (19.8-34.1)*	32.2 (25.3-42.5)*†	<0.001
Waist circumference (cm)	85.5 (71.0-109)	93.2 (79.0-108)	112 (94.0-135)*†	<0.001
Heart rate (bpm)	61 ± 10	61 ± 8	71 ± 14*	0.004
Systolic blood pressure (mmHg)	115 ± 9	123 ± 13	133 ± 16*	<0.001
Diastolic blood pressure (mmHg)	75 ± 6	80 ± 10	88 ± 10*	<0.001
Total cholesterol (mmol/l)	4.21 ± 0.80	5.08 ± 0.94*	5.10 ± 0.63*	<0.001
LDL cholesterol (mmol/l)	2.40 ± 0.69	3.16 ± 0.88*	3.04 ± 0.53*	0.001
HDL cholesterol (mmol/l)	1.47 ± 0.43	1.35 ± 0.40	1.00 ± 0.26*†	<0.001
TGs (mmol/l)	0.82 (0.35-1.30)	0.92 (0.41-5.13)	2.50 (0.89-6.26)*†	<0.001
Apolipoprotein B (mg/dl)	72 ± 16	94 ± 28*	117 ± 26*†	<0.001
Fasting FFAs (µmol/l)	429 ± 209	529 ± 191	500 ± 163	0.175
fP-ALT (U/l)	24 (7-59)	25 (8-98)	38 (20-259)*†	<0.001
fP-glucose (mmol/l)	5.0 (4.5-5.9)	5.3 (4.4-6.5)	5.8 (4.9-6.9)*†	<0.001
fS-insulin (mU/l)	2.5 (0.9-5.7)	4.3 (1.3-22.5)*	11.6 (3.3-36.9)*†	<0.001
HOMA-IR index	0.6 (0.2-1.5)	1.0 (0.3-5.1)*	3.0 (0.8-8.0)*†	<0.001
Myocardial TG content (%)	0.38 (0.14-1.00)	0.72 (0.21-1.39)*	1.11 (0.35-2.33)*†	<0.001
Epicardial fat (mm^2)	489 (283-807)	692 (251-1750)*	930 (565-1666)*†	<0.001
Pericardial fat (mm^2)	527 (66-1280)	1170 (261-6130)*	1940 (615-4631)*†	<0.001
Visceral fat (cm^3)	521 (67-2280)	1890 (217-4180)*	3910 (1257-5740)*†	<0.001
Subcutaneous fat (cm^3)	1460 (381-880)	2840 (284-6200)*	5050 (2216-9350)*†	<0.001
VAT/SAT ratio	0.38 (0.13-1.09)	0.60 (0.28-1.31)*	0.65 (0.38-1.64)*	<0.001
LV ejection fraction (%)	63 ± 4	61 ± 4	60 ± 7	0.172
LV ESV/BSA (ml/m^2)	32 ± 7	29 ± 6	26 ± 6	0.123
LV SV/BSA (ml/m^2)	53 ± 9	45 ± 7	39 ± 9*	<0.001
LV EDV/BSA (ml/m^2)	85 ± 14	74 ± 13	65 ± 13*	0.001
LV mass /BSA (g/m^2)	64 ± 7	59 ± 8	58 ± 9	0.211
Peak filling rate (ml/s)	699 (459-1315)	505 (329-980)	471 (238-909)*	0.005
PFR/LV EDV (s^{-1})	4.07 (3.03-7.23)	3.48 (2.56-5.58)	3.17 (1.89-5.46)*	0.003
LV early diastole (%)	78 ± 9	73 ± 10	68±9*	0.033

Data are expressed as means (±SD) or medians (range).
P values are presented for ANOVA or ANCOVA between hepatic TG content groups.
*Differs significantly (*P*<0.05) from hepatic TG content group low
†Differs significantly (*P*<0.05) from hepatic TG content group moderate. Tests are Bonferroni adjusted for multiple comparisons.

High hepatic TG content and high amount of visceral fat associate with LV diastolic dysfunction (III)

In age-adjusted analyses, parameters of LV diastolic function showed a stepwise decrease over tertiles of liver fat (Table 4). PFR, PFR/LVEDV, and ratio of LV early diastole differed significantly between the high and low liver fat groups. All parameters behaved similarly when the subjects were categorized based on VAT (III, Fig. 2; Tables 1 and 2 in the Data Supplement). This was due to high interrelationship of hepatic TG and VAT ($r=0.832$; $P<0.001$). LV EF, LV mass, and LV ESV/BSA were similar between liver fat tertiles. Subjects with high liver fat had decreased LV EDV and SV indexed to BSA compared with low liver fat subjects.

In univariate correlation analyses adjusted for age, BMI, smoking, heart rate, and blood pressure parameters (III, Table 3), LV PFR and PFR/LVEDV were inversely correlated with hepatic TG and VAT demonstrating LV diastolic dysfunction. Furthermore, PFR was inversely correlated with the VAT/SAT ratio.

Hepatic steatosis and visceral fat are strong predictors of LV diastolic dysfunction (III)

We performed multivariable regression analyses with diastolic function parameters as independent variables and different ectopic fat depots as dependent variables (III, Table 4). Due to high interrelationship, visceral fat and liver fat were not forced into same model. Analyses were adjusted for age, BMI, waist circumference, blood pressure parameters, heart rate, smoking status, TGs, HDL, and fasting plasma glucose. The analyses revealed that hepatic TG was the best predictor of LV PFR and PFR/LVEDV (model 1). The results of multivariable analyses were closely similar when hepatic TG was replaced with VAT (model 2). When the effect of hepatic or visceral fat was considered, there was no significant association between myocardial TG and diastolic parameters. However, pericardial fat appeared to be the best predictor of ratio of LV early diastole.

MetS associates with LA dysfunction (IV)

LA CMR data is outlined in Table 5. LA ED (maximum) areas (2D model) and volumes (3D model) were comparable between study groups. LA ES (minimum) areas and volumes were larger in the MetS group than in control group. However, BSA-indexed LA ED or ES areas (2D model) or volumes (3D model) did not differ between groups. In both 2D and 3D models, LA EF was lower in the MetS group than in the group without MetS indicating LA dysfunction.

LA dysfunction is associated with several contributory factors of MetS (IV)

In both 2D and 3D models of LA EF, age, waist circumference, BMI, fasting plasma glucose, fasting serum insulin, HOMA-IR index, hs-CRP, hepatic TG content, VAT, and SAT correlated inversely with LA EF and HDL correlated positively with LA EF (IV, Table 2 and Fig. 1). In the 3D LA model, correlations of myocardial TG content, epicardial and pericardial fat with LA EF were not significant (Fig. 9).

Univariate linear regression analysis was performed for the 3D model of LA EF (IV, Table 3). In age-adjusted linear regression model, regression coefficients with LA EF and waist circumference, BMI, SAT, VAT, fasting serum insulin, HOMA-IR index, and HDL were significant. Negative correlation of LA EF and glucose, hs-CRP, and hepatic TG content existed but these did not remain significant after adjusting for age.

Table 5. *Summary of left atrial dimensions and function.*

	MetS present	**MetS absent**	***p***
N	33	40	
2D model			
LA 3ch Area EF (%)	32.1 ± 6.3	36.8 ± 8.4	0.008
LA 3ch Area ED (mm^2)	2230 ± 470	1980 ± 320	0.012
LA 3ch Area ED/BSA (10^{-4})	10.2 ± 2.0	10.1 ± 1.6	0.786
LA 3ch Area ES (mm^2)	1520 ± 350	1250 ± 270	0.001
LA 3ch Area ES/BSA (10^{-4})	6.9 ± 1.5	6.3 ± 1.3	0.095
3D model			
LA EF (%)	44 ± 7.7	49 ± 8.6	0.013
LA EDV (ml)	86 ± 23	79 ± 17	0.160
LA EDV/BSA (ml/m^2)	39 ± 9.7	40 ± 8.5	0.638
LA ESV (ml)	48 ± 15	40 ± 11	0.015
LA ESV/BSA (ml/m^2)	22 ± 6.5	20 ± 5.1	0.285

Data are expressed as means (± SD). MetS, metabolic syndrome; LA, left atrial; EF, ejection fraction; EDV, end-diastolic volume; ESV, end-systolic volume; BSA, body surface area.

Visceral fat is the best predictor of LA dysfunction (IV)

Stepwise multivariable regression analysis was performed to further examine the relationship of ectopic fat depots and LA EF (IV, Table 4). It revealed that VAT followed by SAT were the best predictors of reduced LA EF when age, BMI, and hepatic TG content were taken into account.

Three-chamber prolate ellipsoid method is a feasible method for measuring LA volume (IV)

Comparison of LA volume assessment by 3ch prolate ellipsoid method and SA volumetric method was performed. LA measurements obtained with 3ch-based volume assessment correlated highly ($r = 0.97$ for EDV, $r = 0.90$ for ESV, and $r = 0.93$ for EF, $p<0.001$ for all correlations) with volumetric SA-based Simpson's method. Bland-Altman analysis (IV, supplemental Fig. 3) demonstrated mean difference of LA EDV -1.06 ±3.60 ml, LA ESV -1.54 ±3.12 ml and EF 0.83 ±3.16 %.

DISCUSSION

In this study, we used MRI and ^{1}H-MRS to examine cardiac steatosis by quantifying all three cardiac fat stores and cardiac function and related these data to intra-abdominal fat depots, including liver fat, in a relatively large nondiabetic group of abdominally obese men free of clinical CVD. The definition of MetS was used as a tool to identify obese subjects at high cardiometabolic risk in this study.

Ectopic fat deposition in MetS

In MetS, all examined ectopic fat depots were increased compared to controls. We found an ~2-fold elevation in myocardial and epicardial fat, and an ~3-fold elevation in pericardial fat depot in subjects with MetS. Myocardial TG content correlated with visceral fat, hepatic TG content, and multiple parameters of overall obesity. The association of multiple cardiometabolic risk factors with myocardial TG content, pericardial fat, and epicardial fat agrees with previous studies [77, 139].

Most studies have measured either pericardial or epicardial fat depots because their quantification is technically easier than that of lipids in cardiomyocytes, the latter requesting a more sophisticated technique. Overall, both pericardial and epicardial fat depots associate with the amount of visceral fat that is increased in obesity, subjects with MetS, impaired glucose tolerance, and subjects with type 2 diabetes mellitus [139]. Notably, these 3 cardiac sites differ in their capacity to accumulate fat, with the pericardial site having the greatest capacity and cardiomyocytes having the lowest capacity. Thus, growing of cardiac fat depots in men with MetS in context of visceral adiposity is an expected finding.

Our data confirm and expand the results by Gaborit et al. [140], who reported that epicardial fat volume and myocardial TG content were increased in individuals with MetS. Notably, the 2 studies show differences in participants because the cohort of Gaborit et al. [140] exhibited extreme obesity (BMI range, 33 to 52 kg/m^2), including only 18 subjects with MetS, and reported no data on hepatic TG content. Furthermore, our results expand previous observations of increased myocardial lipid accumulation in insulin-resistant states in subjects with impaired glucose tolerance and T2DM to abdominally obese men with cardiometabolic risk factors [84-86]. We showed that visceral fat was an independent predictor of TGs, HDL, and measures of glucose metabolism, on the contrary to the subcutaneous fat which was related to blood pressure parameters. The data highlight that visceral adiposity lies at the root of the systemic effects linked to ectopic fat depots quantified by MR technology, as proposed also by Després [5].

The inefficient storage capacity of SAT mass combined with extra caloric intake results in excessive flow of FFAs into the systemic circulation, driving the overload of FFAs into ectopic fat depots [5]. In this concept, expansion of visceral, pericardial, and epicardial fat depots represent visceral components and the preferential places to store excess dietary fats. Our data support the concept that visceral fat depot is the priority site for excess fat storage. Moreover, pericardial and epicardial fat depots are interrelated with visceral fat and good markers of ectopic fat deposition.

The constellation of these 3 fat depots is linked to the development of the cardiometabolic risk profile, including disturbances of both glucose and lipid metabolism.

The liver plays a central role in controlling lipid and FFA metabolism. Notably, the liver is exposed to first-pass FFAs from VAT. Recognizing the strong correlation between VAT and hepatic TG content, it was not surprising that hepatic TG content showed a correlation with myocardial TG content. Likewise, McGavock et al. reported a modest correlation between hepatic and myocardial TG content [86].

LV diastolic dysfunction and concentric remodeling in MetS

In our study, MetS was strongly associated with LV diastolic dysfunction, as demonstrated by lowered LV early diastolic PFR and ratio of early diastole. In earlier studies, reduced PFR in insulin-resistant obese women and in T2DM patients have been reported [87, 141]. Rider et al. reported similar findings in a female-dominant gender-mixed cohort consisting of markedly obese subjects (BMI 38.7) with unknown status of MetS [142]. Thus, our study adds to previous knowledge on the association between MetS and LV diastolic dysfunction in non-diabetic male subjects with moderately increased BMI. Additionally, we showed that LV diastolic function gradually decreases across tertiles of increasing VAT and hepatic TG content. Our results give further support to the role of cardiometabolic effects of visceral obesity in the development of obesity-associated LV diastolic dysfunction. Consistent with our findings, visceral obesity, MetS, and T2DM have been related to LV diastolic dysfunction as assessed by feature-tracking CMR in two latter publications. 1) Rayner et al. demonstrated that increasing visceral fat associates with lower peak diastolic strain rate [143]. 2) Ladeiras-Lopes et al., reported that subjects with MetS or T2DM showed lower end-diastolic strain rate and higher strain rate index than individuals without MetS [144].

The LV mass was similar in our two study groups, but LV concentric remodeling was present only in subjects with MetS. Concentric LV remodeling has been associated with abdominal obesity and is considered as an early sign of obesity-related cardiac remodeling before the development of LV hypertrophy [58]. Furthermore, LVGFI has recently been introduced as a novel method to integrate LV structure with global function [132]. An LVGFI value of <37% was associated with a significant risk of cardiovascular events. In our study population, LVGFI was significantly lower in the MetS patients than in the subjects without MetS, also supporting the tenet that the nature of cardiac remodeling is concentric rather than eccentric in MetS.

Further support to these findings has been published recently. Neeland et al. reported that increased central adiposity was independently associated with concentric LV remodeling as measured by increased LV wall thickness, increased LV mass/volume ratio, and smaller LV EDV [145]. Moreover, overall body fat was associated with higher cardiac output and lower systemic vascular resistance, however, this association was not uniform across fat depots. In contrast, a significantly lower cardiac output and higher systemic vascular resistance associated with visceral fat. This was in line with our findings where stroke volume was lower in the MetS group than in controls. They also reported that lower body

(gluteal-femoral) adiposity was associated with eccentric remodeling (increased LV EDV with reduced LV mass, concentricity, and wall thickness) and a higher cardiac output and lower systemic vascular resistance. These findings can be explained by the gender-specific differences in LV remodeling in obesity, as reported by Rider et al. [146]. According to their study, obese men showed predominantly concentric hypertrophy, whereas obese women exhibited both eccentric and concentric hypertrophy. Association of visceral obesity and LV concentric remodeling has also been reported in several later large population MRI studies [147-149]. However, these studies did not report LV diastolic function.

The role of epicardial and pericardial fat in LV function

We found that MetS is strongly associated with an increased amount of VAT which in turn is the best predictor of pericardial and epicardial fat. An increased amount of epicardial fat is related to burden of atherosclerotic plaques, CAD and myocardial ischemia [150, 151]. Epicardial fat as well as pericardial and myocardial fat all correlated to LV concentric remodeling. Furthermore, our CMR study showed that epicardial fat is associated with LV diastolic dysfunction. This is in line with earlier findings based on echocardiography and computed tomography [92]. However, when the effect of visceral or hepatic fat was considered, epicardial fat did not remain as an independent predictor of LV diastolic dysfunction. Therefore, the association of epicardial fat with impaired LV diastolic function is probably indirect due to its close correlation with both hepatic and visceral fat depots.

Most studies have focused on epicardial adipose tissue, and a lesser role has been left to pericardial or intrathoracic fat. In our study, pericardial fat correlated with diastolic dysfunction even on a larger scale of parameters than epicardial fat. It was the best marker of one of the three examined LV diastolic parameters, ratio of early diastole, even when the effect of visceral and hepatic fat was considered. Similarly, as a marker of ectopic fat accumulation, pericardial fat has been reported to associate with insulin resistance and 10-year CAD risk more strongly than epicardial fat [152]. Pericardial fat depot may reach considerable dimensions with obesity, thus representing a mechanical challenge for the beating heart and affecting cardiac dimensions and workload. While epicardial fat has shown to be an active endocrine organ, the role of pericardial fat as a source of proinflammatory or adipokine bioactive molecules is uncertain [95].

Role of liver fat on LV function

Our data revealed that LV diastolic dysfunction was associated with high liver fat content, while systolic function remained unaltered. In contrast, Perseghin et al. reported that LV morphological features were similar between high and low liver TG content [127]. After our study and using comparable methodology, however, results of a large (n=714) population-based study associated hepatic TG content with LV diastolic dysfunction giving further support to our findings [153]. Recently, association of NAFLD or NASH and LV diastolic dysfunction has also been reported in several echocardiographic studies [154-157]. Notably, the changes may be reversible as shown in a recent CMR and ^{1}H-MRS study, where 12 weeks high-

intensity interval training program led to decrease in hepatic TG content and improvement of LV diastolic function [158].

Furthermore, our study showed that ectopic fat in all three cardiac sites accumulated with increasing amount of both hepatic TG and VAT. Our study suggests that visceral adiposity and hepatic fat lie at the root of the systemic harmful effects and LV diastolic dysfunction. The data provide further evidence for the model of two classes of ectopic fat depots with either systemic or local deleterious effects on cardiovascular health [5]. Thus, both NAFLD and VAT seem to influence CVD risk by associating with both metabolic risk and LV diastolic dysfunction.

The pathophysiological mechanisms linking NAFLD with cardiac complications contain a variety of derangements from subtle subcellular changes to systemic changes including hemodynamic, metabolic, hemostatic, hormonal, and cytokine abnormalities, as well as dysregulation in multiple organ systems [120]. These processes are not completely understood and reach beyond the scope of this thesis.

LA dysfunction in MetS

We discovered that in MetS, LA function is decreased as measured by LA EF, without the enlargement of LA. The decrease in LA EF indicates the presence of LA mechanical remodeling, an entity with increasing interest in the recent literature [74, 159]. When LA measurements were compared to patient outcomes, LA EF was superior and incremental to LA volume regarding the assessment of mortality risk in general population [160]. Lower LA EF has also been associated with a poorer prognosis in patients with HF [161] and non-ischemic cardiomyopathies [162]. LA remodeling can be reversible, especially at early stages, and may be treated with medications such as angiotensin-converting enzyme inhibitors or angiotensin II receptor antagonists [163].

In our study, except for blood pressure, all other components (waist circumference, insulin resistance and dyslipidemia (low HDL)) of MetS were related to LA dysfunction. Waist and VAT were better predictors of LA dysfunction than BMI, thus emphasizing the role of visceral obesity in LA remodeling. In a recent population-based study, LA enlargement was associated with visceral obesity, but LA function was not evaluated [164]. In our study, liver fat was also associated with lower LA EF, but the correlation did not remain significant after adjusting for age.

Insulin resistance is a key component of MetS associated with several pathogenetic abnormalities that lead to HF and increased cardiovascular mortality [45]. In our non-diabetic study population, insulin resistance was associated with LA dysfunction as shown by inverse correlation of blood glucose and insulin levels, and HOMA-IR index with LA EF. This is consistent with an earlier MRI study reporting an association of T2DM and lowered LA EF [165].

Obesity and MetS are well-known risk factors of AF and decreased LA EF has been shown to increase the risk for AF independent of LA size [161, 166]. Functional LA remodeling is one of the causes suggested as a factor preceding AF. Evaluating LA function with a relatively simple 3ch CMR method that we

introduced may hence provide valuable clinical information to find particularly those obese persons at risk for developing AF or other cardiovascular events.

Myocardial TG content and cardiac energetics

Interestingly, myocardial TG content was not independently associated with LV diastolic dysfunction when other cardiac fat deposits or hepatic TG or VAT were considered. In line with our results, two studies in insulin-resistant women, both without an assessment of epi/pericardial fat, reported the link between the myocardial TG content and diastolic dysfunction as of borderline significance [141] or negative [167]. Our findings challenge the concept of myocardial lipid accumulation as an unambiguous marker of diastolic dysfunction, as suggested by earlier studies in T2DM patients where myocardial TG content was associated with impaired LV diastolic function [87, 88]. Likewise, McGavock et al. failed to demonstrate any relationship between the degree of myocardial TG accumulation and diastolic function in patients with T2DM [168]. In contrast to our findings, Banerjee et al. reported recently a positive correlation between LV diastolic dysfunction and myocardial TG content in mixed-gender cohort [169]. However, they did not measure epi- or pericardial fat or hepatic TG content, or report whether the finding was independent of VAT.

In our subjects with MetS, myocardial TG content was, however, increased up to two-fold compared with the subjects without MetS. Notably, the range of myocardial TG content (0.14-2.33%) was relatively narrow. Rather than a stable fat deposit, myocardial TG is a highly dynamic lipid pool, where up to three or four-fold increase has been reported following 48–72 h fasting in lean subjects [83, 170]. On the other hand, short-term lipid excess did not increase TG content in cardiomyocytes [82]. In diabetic subjects, 16 weeks calorie restriction decreased myocardial TG levels and improved diastolic function [170]. Hence, myocardial TG content may be adaptive to both short and long-term dietary interventions, and it may thus serve as a relatively rapidly changing reservoir of energy, in an analogous fashion to intramyocytic TG of skeletal muscle [171].

Considering this, the effects of weight loss on myocardial TG content has reported to be surprisingly weak. Gaborit et al. reported that during 6 months follow up, bariatric surgery in morbidly obese patients had no effect on myocardial TG content, although LV diastolic function was improved [172]. Similarly, diet intervention and moderate weight loss over 24 months improved LV remodeling but did not alter myocardial TG levels in overweight or obese postmenopausal women [173].

In our study population, myocardial steatosis was not a key factor of LA dysfunction, although an association of myocardial TG content with reduced LA EF in 2D model was noted. To our knowledge, no earlier ^{1}H-MRS studies exist where myocardial TG content has been examined in atrial dysfunction or AF. For the atria, energy substrate preference has not been investigated but it may not differ from that in the ventricles [174].

The study design does not allow us to evaluate the molecular mechanisms underlying cardiac steatosis. While ^{1}H-MRS is a powerful method for detecting abnormalities in lipid storage, it cannot differentiate between disturbances in lipid

uptake on one hand and lipid utilization on the other. Therefore, the accumulation of intramyocardial TG may result from the imbalance of FFA uptake and oxidation in the heart. Heart and liver share the unique position of first-pass organ being exposed directly to FFA flux from the visceral fat depots. Other potential sources for FFAs are lipolysis of circulating lipoproteins by lipoprotein lipase, hydrolysis of intramyocardial TG by adipose TG lipase, and uptake of lipids via VLDL receptors [175-177]. Labbé et al. used dynamic positron emission tomographic imaging of both heart and liver to follow-up the fate of dietary fatty acids in humans [178]. They demonstrated that obese subjects with impaired glucose tolerance displayed both increased fatty acid uptake and oxidation of postprandial dietary fatty acids. Thus, the oversupply of fatty acids from dietary fat can also be a significant source for intramyocardial fat.

Normal heart utilizes FFAs and glucose as main energy sources with a ratio of 3:1 [179]. However, the heart is constantly switching between glucose and fatty acid metabolism in response to prevailing metabolic and exercise conditions [95]. Therefore, maintaining metabolic flexibility is essential for the preservation of a normal cardiac function. In the setting of obesity, the energy balance is shifted even more from glycolysis toward the increased β-oxidation of fatty acids [180]. Under aerobic conditions, fatty acid oxidation yields more adenosine triphosphate (ATP) per gram of substrate but takes 10-15% more O_2 per amount of ATP than glucose [174]. Therefore, during chronic hypoxia and HF, ventricular metabolism shifts from fatty acid to glucose utilization. Accordingly, we reported that in dilated cardiomyopathy, myocardial TG is markedly lower than in control subjects with comparable abdominal fat distribution [181]. Recently, Rayner et al. reported a progressive decrease in PCr/ATP ratio across the increasing VAT quartiles indicating disturbed myocardial energetics [143]. In addition, peak diastolic strain rate behaved similarly, indicating LV diastolic dysfunction. The diastolic phase of the cardiac cycle is more sensitive to energetic depletion than systolic phase due to the higher energy demands of active calcium uptake. Therefore, it is logical that compromises in myocardial energetics in MetS are linked to LV diastolic dysfunction [143].

In MetS, the increased intramyocardial TG may reflect not only the passive storage but also the enhanced demand of lipids due to the preference of using fat more over glucose as the primary fuel of the heart. Theoretically, myocardial TG content may, however, act ambiguously in obesity-related cardiac dysfunction, as it may increase due to accelerated FFA uptake and β-oxidation, and decrease in response to the higher energy demand, oxidative stress, and increased glycolysis.

In conclusion, understanding of the regulatory processes behind these changes in cardiac substrate selection and utilization remain unclarified and the dynamical role of intramyocytic lipids will merit further studies. As a future research interest, ^{31}P-MRS could be combined with ^{1}H-MRS to further examine the derangements in cardiac energy balance in obesity and MetS.

Study limitations

Our study population (n= up to 77) was relatively large for the meticulous pattern of modern imaging studies. Yet, in terms of assessment of independent predictors of LA and LV dysfunction, the sample size was quite small due to high interrelationship of ectopic fat depots and components of MetS. To exclude the effects of hormonal variability, our study population was limited to men which was a clear limitation of the study. However, gonadal steroids play a major role in the regulation of adipose tissue distribution and consequently have an impact on gender-specific differences in LV remodeling in obesity [31, 146]. Subjects with MetS were older than controls producing a potential source of bias for the evaluation of cardiac steatosis and cardiac dysfunction. Still, our results remained significant after adjusting for age. There was a clear difference in biomarkers between subjects with and without MetS, however, the differences other than MetS criteria might be significant confounders in terms of differences in LV structure and function.

A limitation of ^{1}H-MRS methodology was the narrow range of myocardial TG content in healthy subjects which has been reported to be less than or about 1% [182, 183]. Accordingly, myocardial TG content was low in most lean subjects and this may underestimate its association with metabolic parameters as compared to pericardial fat. Whether the measurement of myocardial TG content from the ventricular septal wall corresponds to TG content of the LA myocardium is unclear, limiting the investigations of cardiac steatosis in LA dysfunction.

Although the measurements of diastolic function consisted of multiple parameters, they were based on LV short-axis cine images. Other methods, such as trans-mitral velocity-encoded flow analysis, might have served as an internal reference. However, pseudonormal E/A-pattern related to diastolic dysfunction might be a pitfall of this technique. In our experience, velocity measurements are easily subject to averaging errors because of data gathering time of several minutes and intracycle variation.

Another source of bias is that the measurement of LA volume did not cover the whole volume of LA but was based on a single image plane of 3ch images with the third dimension taken into account by measuring the LA diameter in perpendicular SA plane. Absolute LA volumes correlated well with those measured from SA images with Simpson's method considered as gold standard in our small study population, and the absolute LA volume values we obtained were comparable to earlier CMR and multidetector computed tomography studies calculated with Simpson's method [184-186]. Phasic LA function analysis could have been informative to further evaluate the mechanism of atrial dysfunction and remodeling.

Finally, the cross-sectional nature of the study design limits inferences of causality.

CONCLUSIONS

1. In MetS, myocardial TG content is increased but is also influenced by other factors than the excess visceral obesity, e.g. by altered energy metabolism. The best independent predictor of cardiometabolic risk factors is VAT.

2. MetS is associated with LV diastolic dysfunction and concentric LV remodeling. Myocardial TG content is, however, not an independent predictor of LV diastolic dysfunction.

3. Epicardial and pericardial adipose tissue as well as myocardial TG content accumulate with increasing amount of both hepatic TG content and VAT. Hepatic steatosis and VAT are associated with LV diastolic dysfunction.

4. MetS is associated with subclinical LA dysfunction without enlargement of the LA. Moreover, LA dysfunction was associated with several contributory factors of MetS including waist circumference, insulin resistance, dyslipidemia, and VAT. The best independent predictor of LA dysfunction is VAT. Notably, the role of myocardial TG remains inconclusive.

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